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(54) Title: DETECTION OF ANALYTES BY FLUORESCENT LANTHANIDE CHELATES
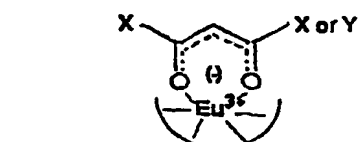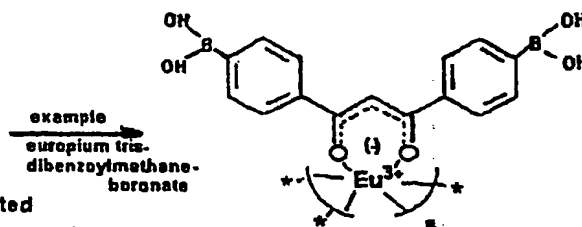
(57) Abstract

Compositions and methods for determining the presence or concentration of an analyte in a sample by exposing the sample to an indicator molecule comprising a fluorescent lanthanide metal chelate complex. The presence or concentration of the analyte in the sample is determined by observing and/or measuring the change in intensity of fluorescence emitted by the lanthanide metal chelate complex upon binding of the analyte to one or more recognition elements in the complex. The fluorescent indicator molecules can be used in various types of fluorescent sensing devices and are useful in various fields, including energy, medicine and agriculture.

TITLE OF THE INVENTION

5   DETECTION OF ANALYTES BY FLUORESCENT LANTHANIDE CHELATES

CROSS-REFERENCE TO RELATED APPLICATIONS

10   This application is a continuation-in-part of
application Serial No. 09/037,960 filed March 11, 1998.

STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH OR DEVELOPMENT

15   Not applicable.

BACKGROUND OF THE INVENTION
1.   Field of the Invention
          This invention relates to fluorescent compounds
20   useful as indicator molecules for detecting the presence
or concentration of an analyte in a medium, such as a
liquid, and to methods for achieving such detection.
More particularly, the invention relates to fluorescent
lanthanide metal chelate complexes containing substituted
25· ligands and their use as indicator molecules for
detecting the presence or concentration of an analyte
such as glucose or other cis-diol compound in a medium,
including a liquid medium such as a biological fluid.

30   2.   Description of the Related Art
          Certain rare-earth metal chelates emit visible light
upon irradiation with UV light and different forms of
visible light (e.g., violet or blue light), an emission
which is characterized by the chelated cation. Some
35   lanthanide ions, such as those of europium (Eu^{3+}),

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samarium (Sm^{3+}), terbium (Tb^{3+}), and to a lesser extent dysprosium (Dy^{3+}) and neodymium (Nd^{3+}), exhibit typical fluorescence characterized by the ion, especially when chelated to suitable excitation energy mediating organic

5   ligands. The fluorescent properties of these compounds -- long Stokes' shift, narrow band-type emission lines, and unusually long fluorescence lifetimes -- have made them attractive candidates for fluorescent immunoassays and time-resolved fluorometric techniques.

10   The major emission lines of these fluorescent lanthanide chelates are formed from a transition called hypersensitive transition and are around 613-615 nm with Eu^{3+}, 545 (and 490) nm with Tb^{3+}, 590 and 643 nm with Sm^{3+}, and 573 with Dy^{3+}. See Hemmila, Application of

15   Fluorescence in Immunoassays, 140-42 (1991). See also Spectroscopy in Inorganic Chemistry, vol. 2, at 255-85 (Academic Press 1971). Radiation is typically absorbed by the chelates at a wavelength characteristic of the organic ligand and emitted as a line spectrum

20   characteristic of the metal ion because of an intramolecular energy transfer from the ligand to the central metal ion. The organic ligand absorbs energy and is raised or excited from its singlet ground state, S_0, to any one of the vibrational multiplets of the first

25   singlet excited state, S_1, where it rapidly loses its excess vibrational energy. At this point, there are two possibilities: relaxation by an $S_1 \to S_0$ transition (ligand fluorescence) or intersystem crossing to one of the triplet states, T_1. See E.P. Diamandis et al., Analytical

30   Chemistry 62:(22):1149A (1990); see also Spectroscopy in Inorganic Chemistry, vol. 2, at 255-85 (Academic Press 1971).

Fluorescent europium chelates are known to exhibit large Stokes shifts (~290 nm) with no overlap between the

35   excitation and emission spectra and very narrow (10-nm

bandwidth) emission spectra at 615 nm. In addition, the
long fluorescence lifetimes (measurable in microseconds
instead of the nanosecond lifetimes measurable for
conventional fluorophores) of the chelates help filter

5   out noise and other interference having a low fluorescent
lifetime. The long fluorescent lifetimes thus permit use
of the chelates for microsecond time-resolved
fluorescence measurements, which further reduce the
observed background signals. Additional advantages of

10   using europium chelates include that europium chelates
are not quenched by oxygen.

       Line emissions of two europium (Eu) chelates, Eu-
dibenzoylmethide and Eu-benzoylacetonate, have made the
chelates attractive candidates for use in lasers. <u>See</u> H.

15   Samuelson, et al. (<u>J. Chem. Physics</u> 39(1): 110-12 (1963))
Samuelson, et al. studied the fluorescence and absorption
of the above two europium chelates as solids and in
solution. Samuelson, et al. compared the fluorescent
lifetimes of the europium chelates under various

20   conditions with the lifetimes of europium fluorescence in
other compounds. Based on this comparison, Samuelson, et
al. suggested that the variation in lifetimes between the
two groups of europium compounds is a result of the
ligand-Eu interaction in the europium chelates.

25   Specifically, Samuelson et al. determined that various
emission lines from Eu-dibenzoylmethide showed
fluorescent lifetimes of 480 +/- 50 μs, which were
significantly greater than the fluorescent lifetimes in
other europium compounds.

30   Crosby, et al., <u>J. Chem. Physics</u> <u>34</u>:743 (1961) had
previously studied the role of intramolecular energy
transfer in sensitizing ion emission from rare-earth
metal chelates, including europium dibenzoylmethide and
europium benzoylacetonate chelates. Whan, et al., <u>J.</u>

35   <u>Mol. Spectroscopy</u> <u>8</u>: 315-27 (1962) reported that the

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emission from chelates of a group of lanthanide metal
ions (Eu^{3+}, Tb^{3+}, Dy^{3+} and Sm^{3+}) was dominated by bright
spectral lines characteristic of the individual rare-
earth metal ions. Whan, et al. found that both the
5   benzoylacetonates and dibenzoylmethides of Eu^{3+} and Tb^{3+}
are especially bright emitters and that the bright line
emissions and low yields of phosphorescence from these
chelates indicated that intramolecular energy transfer
from the ligands to the Eu^{3+} and Tb^{3+} ions of these
10 chelates occurs efficiently. Whan, et al., at 324.

N. Filipescu, et al., J. Physical Chem. 68(11):3324
(1964) reported that the fluorescence spectra of europium
and terbium β-diketone chelates are modified when
substituents are changed in the organic ligand portion of
15 the chelates. Filipescu, et al. discussed the relative
intensity, spectral distribution, shifting, and splitting
of the fluorescence lines of the europium and terbium
chelates in relation to the nature of substituents, their
position, molecular configuration, and the overall
20 intramolecular energy transfer. Filipescu, et al. found
that the overall fluorescence intensity characteristic of
the ion depended on two factors: 1) the amount of energy
available at the organic triplet, and 2) the efficiency
of energy transfer to the ion.

25   Filipescu, et al. also found that the above two
factors varied for different substituents. For instance,
the substitution of europium dibenzoylmethide chelates
with electron-donor methoxy groups in the meta position
on the chelate was found to enhance the fluorescent
30 emission of the europium ion, whereas paramethoxy
substitution was found to decrease the europium
fluorescence. Additionally, the effect was more
pronounced for the di- than for the monomethoxy-
substituted dibenzoylmethides. In contrast, an opposite
35 effect was observed for nitro-substituted

dibenzoylmethides of europium. The electron-withdrawing
nitro groups attached to the *para* or *meta* positions were
found to decrease the total ionic emission of europium.
Additionally, the effect was more pronounced for di- than
5   for monosubstituted dibenzoylmethides.

    Filipescu, *et al.* further found that the strong ionic
fluorescence emitted by europium *para*-
phenyldibenzoylmethide indicated that increasing the size
of the aromatic system enhanced the amount of energy
10   transferred to the europium ion. This fact was confirmed
by the emission results obtained for napthyl-substituted
diketones which were found to have substantially higher
ionic emissions than the dibenzoylmethide chelates.
Filipescu, *et al.*, at 3328-29.

15   E. Diamandis, *et al.*, <u>Analytical Chemistry</u>
<u>62(22)</u>:1149A (1990), described how europium chelates can
be used as labels in fluorescence immunoassays and DNA
hybridization assays. With respect to fluorescent
immunoasays, the authors described that europium chelates
20   can be used as immunological labels in various assay
configurations, including either competitive or
noncompetitive assays.

    U.S. Patent No. 4,374,120 (Soini, *et al.*) describes a
method for detecting a substance using a fluorescent
25   lanthanide chelate complex as a marker. U.S. Patent No.
4,374,120 also describes the use of β-diketones as
enhancing ligands for promoting the strong fluorescence
properties of certain lanthanide chelates, especially
chelates of europium and terbium.

30   Wallac (Turku, Finland) developed a lanthanide metal
chelate to replace radiation tags for conducting
immunoassays, having the structure:

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$$La^{3+}$$
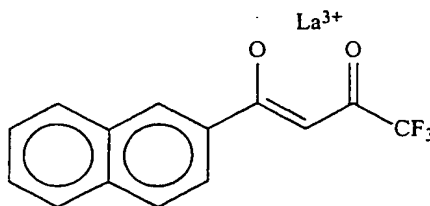
The Wallac molecule was found to behave very efficiently in dilute solutions. See Hemmila, Applications of Fluorescence in Immunoassays, p. 149 (1991).

Certain conditions are required for using lanthanide metal chelates in aqueous solutions, such as in biological fluids. For example, it is known that chelates must, first, be dissolved in the aqueous solution, and second, avoid being quenched by water molecules which tend to fill up the empty coordination sites of the lanthanide ion. However, various adducts or Lewis bases, such as phosphines, phosphine oxides, or nitrogen heterocycles, have been used in addition to the ligand structure to form an "insulating sheet" around the lanthanide ion, enhancing the fluorescence by preventing water molecules from penetrating into the complex's inner sphere. For example, solutions developed for fluorometric determinations of lanthanides in aqueous systems (e.g., immunoassays) have comprised β-diketones and trioctylphosphine oxide ("TOPO") as an adduct forming synergistic agent, and a detergent (e.g., Triton X100) which forms micelles and helps to solubilize the coordinated complex. See Applications of Fluorescence in Immunoassays, at 146-47.

Lanthanide metal chelate complexes have not been previously examined or constructed for the purpose of active detection of an analyte by utilizing a discrete and specific recognition element feature, such as a boronate group for detecting glucose and other cis-diols, through one or more ligands contained in the chelate

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complex. As discussed above, lanthanide metal chelates
have been investigated primarily for use as laser dyes,
substitute labels for radioisotopes, and for attachment
to antibodies as labels in immunoassays. Lanthanide

5   metal chelates also have been used for qualitative
analytical procedures for detecting tetracycline.

Glucose is an organic compound indispensable to
living organisms and plays an important role in
information transmission, energy metabolism and structure

10   formation in such organisms. For example, glucose, and
more particularly, D-glucose, is crucial as an energy
source for a variety of cells in constructing various
organs. Glucose is stored in the liver as glycogen,
which is released in body fluids as needed for energy

15   consumption. The production and consumption of glucose
are well balanced in the body fluids of a normal or
healthy human being, maintaining the glucose
concentration constant in the fluids. Thus, detecting
sub-levels or supra-levels of glucose in the blood or the

20   urine provides valuable information for diagnosing such
diseases as diabetes and adrenal insufficiency.

A glucose sensor using an enzyme (e.g., as made by
Yellow Springs Instruments (YSI), Ohio) is the best known
practical measure for detecting glucose. This technique

25   involves decomposing glucose with an enzyme (glucose
oxidase) and measuring the amount of hydrogen peroxide
produced by the decomposition through an appropriate
means (such as by an electrode). Although this method is
well established, the quality of the enzyme, which

30   originates from a living body, will irreversibly change
over time and cannot be recycled for reuse.
Additionally, because the glucose is actually consumed in
the detection reaction, the intrinsic ability of the
glucose sensor to measure low levels of analyte is

35   therefore limited.

It is well known that boronic acid-containing
compounds bind to glucose. The mechanism is believed to
occur through bonding of adjacent hydroxyl groups on
glucose to hydroxyl groups on a boronate moiety, as drawn
5   below:
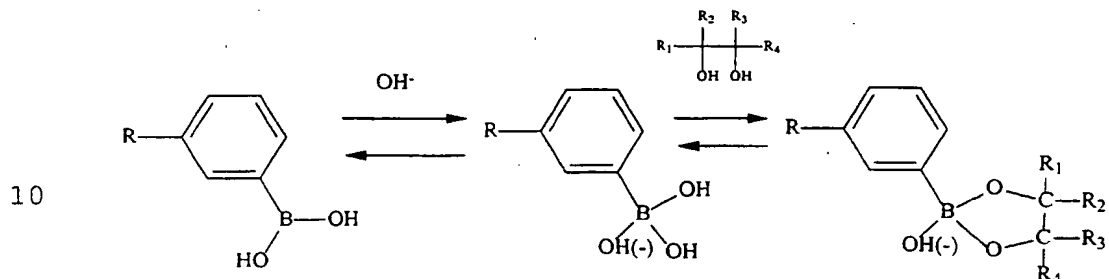
The complexation of carbohydrates, including glucose,
with phenylboronic acid has been known for a long time
15   and the reversibility of that interaction has served as a
basis for the chromatographic separation of sugars.
Specifically, in 1959, Lorand and Edwards reported
association constants for aqueous associations of
phenylboronic acid with many saturated polyols; binding
20   interactions ranged from very weak (e.g., ethylene
glycol, K_d=360 mM) to moderately strong (e.g., glucose,
K_d=9.1 mM). See J. Yoon, et al., Bioorganic and Medicinal
Chemistry 1(4):267-71 (1993).

U.S. Patent 5,503,770 (James, et al.) describes a
25   fluorescent boronic acid-containing compound that emits
fluorescence of a high intensity upon binding to
saccharides, including glucose. The fluorescent compound
has a molecular structure comprising a fluorophore, at
least one phenylboronic acid moiety and at least one
30   amine-providing nitrogen atom where the nitrogen atom is
disposed in the vicinity of the phenylboronic acid moiety
so as to interact intermolecularly with the boronic acid.
Such interaction thereby causes the compound to emit
fluorescence upon saccharide binding. U.S. Patent
35   5,503,770 describes the compound as suitable for

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detecting saccharides. See also T. James, et al., J. Am. Chem. Soc. 117(35):8982-87 (1995).

Additionally, fluorescent sensors using an anthrylboronic acid-containing compound for detecting
5   blood glucose are known in the art. For example, J. Yoon, et al., J. Am. Chem. Soc. 114:5874-5875 (1992) describe that anthrylboronic acid can be used as a fluorescent chemosensor for signaling carbohydrate binding, including binding of glucose and fructose.
10   An object of the present invention is to detect the presence or concentration of an analyte in a medium such as a liquid or gas by measuring any change in fluorescence emitted by a lanthanide metal chelate complex upon binding of the analyte to one or more
15   chelators of the chelate complex through an analyte-specific recognition element.

Another object of the present invention is to provide an analyte-specific, recognition element-containing lanthanide metal chelate complex as an indicator molecule
20   for detecting the presence or concentration of an analyte such as glucose or other cis-diol compound in a medium such as a liquid.

                    BRIEF SUMMARY OF THE INVENTION
25   The present invention is directed to an indicator molecule for detecting the presence or concentration of an analyte, comprising a fluorescent lanthanide metal chelate complex having the formula:

$$M(--Ch(-R_x))_Y$$
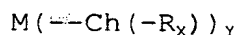
30   wherein:

M represents a lanthanide metal ion; Ch represents a chelator comprising a ligand, preferably an organic ligand which can comprise any one or more of a β-diketone or a nitrogen analog thereof, a dihydroxy, a carboxyl
35   coordinating heterocycle, an enol, a macrobicyclic

                              -9-

cryptand (*i.e.*, a cage-type ligand), a phenylphosphonic acid, or a polyamino-polycarboxylic acid. The organic ligand of Ch can also comprise any one or more of a heterocycle of nitrogen, sulfur, and linked carboxyls.

5   The organic ligand of Ch can further comprise any one or more of an alkane or alkene group, preferably containing 1 to 10 carbon atoms, as well as aromatic, carbocyclic or heterocyclic moieties, including benzyl, napthyl, anthryl, phenanthryl, or tetracyl groups. Furthermore,

10   one or more chelators complexed with M can be the same or a mixture of different chelators (so-called "mixed ligand or ternary chelates").

R represents an analyte-specific recognition element, one or more of which is bound to one or more ligands of

15   the chelate complex, but need not be linked to every ligand of the chelate complex. In a preferred embodiment of the present invention, R can be a boronate group or a compound containing a boronate group for detecting glucose or other cis-diol compound.

20   X represents the number of recognition elements R bound to each of one or more chelators. X can be an integer from 0 to 8, and in certain preferred embodiments of the invention, X=0 to 4 or X=0 to 2. Additionally, the number of recognition elements R bound to each of one

25   or more chelators may be the same or different, provided that for one or more chelators, X>0. Y represents the number of chelators complexed with M, and can be an integer from 1 to 4. In certain preferred embodiments of the invention, Y=1, Y=3 or Y=4.

30   The present invention also is directed to a fluorescent lanthanide metal chelate complex, as defined above.

The present invention further is directed to methods for detecting the presence or concentration of an analyte

35   by utilizing the above indicator molecule and fluorescent

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lanthanide metal chelate complex. The method comprises
the steps of exposing the sample to an indicator molecule
comprising a fluorescent lanthanide metal chelate complex
having the above-defined formula, and measuring any
5   change in fluorescence emitted by the lanthanide metal
chelate complex, and therey detecting the presence or
concentration of the analyte.

In the present invention, the presence or
concentration of the analyte is detected by measuring any
10   change in fluorescence emitted by the lanthanide metal
chelate complex upon binding of the analyte to one or
more chelators of the chelate complex through one or more
analyte-specific recognition elements. Specifically, the
presence or concentration of an analyte, such as glucose
15   or other cis-diol compound, is determined by observing
and/or measuring the change in intensity or lifetime of
fluorescence emitted by the fluorescent metal ion (i.e.,
the fluorescence is attenuated, enhanced or shifted in
wavelength) upon binding of the analyte to the analyte-
20   specific recognition element of the chelate, which for
detecting glucose or other cis-diol compound is a
boronate-containing recognition element.

The present invention offers the advantage of being
able to detect an analyte, such as glucose or other cis-
25   diol compound, in an analyte-specific manner in a medium
such as a liquid or a gas, utilizing a fluorescent
indicator molecule having a fluorescent lifetime of
sufficient length (measurable in microseconds instead of
nanoseconds), as well as having a long Stoke's shift,
30   thereby decreasing the effect of any background noise and
other interference which would reduce the sensitivity of
the analyte detection, and is not concentration quenched.

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the indicator molecule of the invention will be illustrated by reference to the accompanying drawings in which:

5   Figure 1 illustrates a boronic acid-containing europium chelate in accordance with the present invention (shown having only one ligand for purposes of clarity).

Figure 2 also illustrates a boronic acid-containing europium chelate in accordance with the present
10   invention.

Figure 3 illustrates a europium chelate complex containing multiple boronic acid-containing ligands in aqueous solution in the presence of trioctylphosphine oxide ("TOPO").

15   Figure 4 illustrates the effect of adding catechol to an ethanol solution containing a boronic acid-containing europium chelate in accordance with the present invention.

Figure 5 illustrates the effect of catechol addition
20   to an ethanol solution containing europium β-naphthoyltrifluoroacetate (Eu-bNTA).

Figures 6-8 illustrate a Eu-NTA-boronate titration with glucose in methanol.

Figure 9 illustrates a comparison between a europium
25   dibenzoylmethane (Eu(DBM)) and Eu(boronated DBM) titration with glucose and fructose in methanol.

Figure 10 illustrates an Eu-NTA boronate titration with glucose in methanol.

Figure 11 illustrates an Eu-DBM boronate titration
30   with glucose and fructose in methanol.

Figure 12 illustrates an Eu(theonyl-4-benzoylmethaneboronic acid) titration with glucose and fructose in methanol.

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Figure 13 illustrates an Eu(benzoyl-
trifluoromethylacetoneboronic acid) titration with
glucose and fructose in methanol.

5   DETAILED DESCRIPTION OF THE INVENTION
As stated above, the presence or concentration of an
analyte is determined in the present invention by
observing and/or measuring the change in intensity or
lifetime of fluorescence emitted by the fluorescent
10   indicator molecule after binding to an analyte through
one or more analyte-specific recognition elements in the
indicator molecule. The fluorescent indicator molecule
comprises a lanthanide metal chelate complex having the
formula:
15                          $M(--Ch(-R_x))_Y$
wherein:
M represents a lanthanide metal ion; Ch represents a
chelator comprising a ligand, preferably an organic
ligand, which can comprise any one or more of a β-
20   diketone or a nitrogen analog thereof, a dihydroxy, a
carboxyl coordinating heterocycle, an enol, macro-
bicyclic cryptand (i.e., a cage-type ligand), a
phenylphosphonic acid, a cyclen (tetra aliphatic
carboxylates or phosphonates of 1,4,7,10-
25   tetraazacyclododecane) or a polyamino-polycarboxylic
acid. The organic ligand of Ch can also comprise any one
or more of a heterocycle of nitrogen, sulfur, and linked
carboxyls.
R represents an analyte-specific recognition element,
30   one or more of which is bound to one or more ligands of
the chelate complex, but need not be linked to every
ligand of the chelate complex. In a preferred embodiment
of the present invention, R can be a group for detecting
glucose or other cis-diol or cis-diol acting compounds.
35   Such groups include boronates, arsenites and germanates,

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and compounds containing those groups. Representative
boronate-containing compounds include those having the
following general structures (in each structure, R' and
R" are each independently fused aryl; aliphatic; primary,
5   secondary or tertiary amine; amide; carboxyl; ketone;
ester; alcohol; or aldehyde; and Y and Z are each
independently aliphatic, alkoxy or aryl):

10
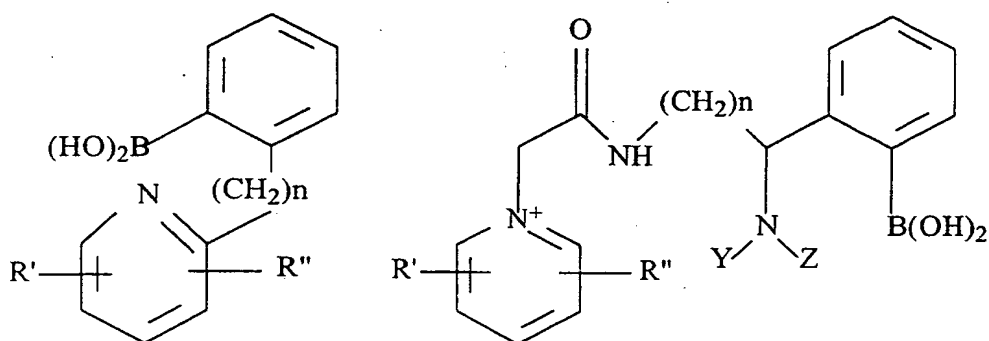
wherein n is 0 or 1 in the left structure and 0, 1 or 2
in the right structure;

15
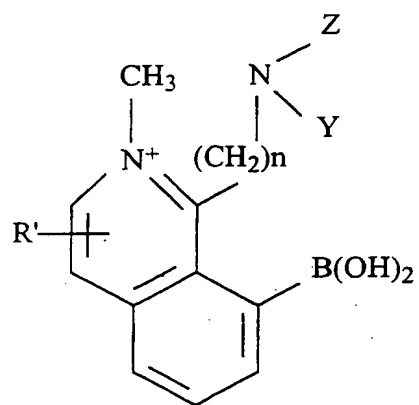
wherein n is 1;

5
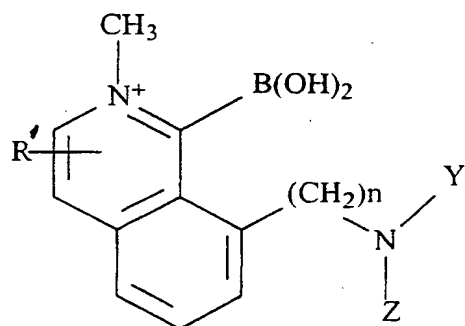
wherein n is 0 or 1;

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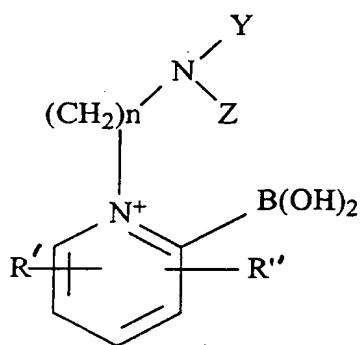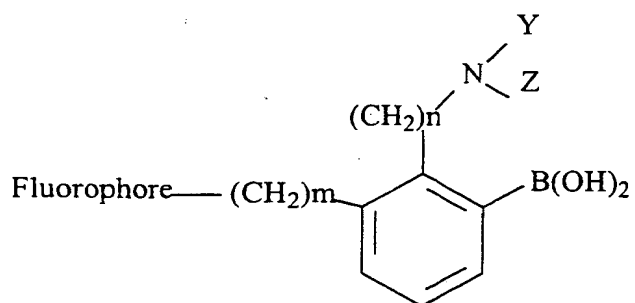
-15-

wherein n is 2;

wherein m is 0-5 and n is 1 or 2; and

-16-
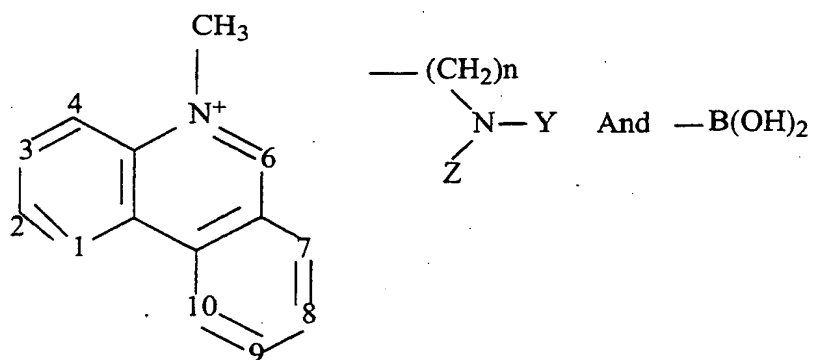
$$\text{CH}_3$$

(structure of a tricyclic ring system with methylated quaternary nitrogen N+, carbons numbered 1–10)

$$-(\text{CH}_2)n \quad \text{And} \quad -\text{B(OH)}_2$$
$$\underset{\overset{|}{Z}}{N}-Y$$

wherein n is 0 or 1, and the boronic acid and amine
substituents are located as a pair on positions 1 and 10,
3 and 4, 6 and 7, 7 and 8 or 9 and 10.

Some specific boronate-containing compounds include:
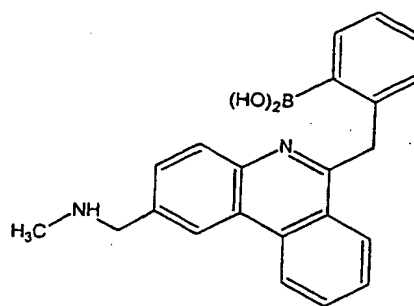
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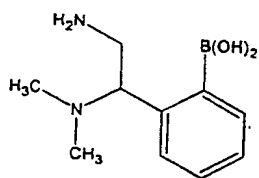
Target B
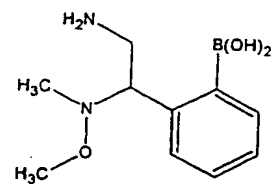
Target B-MeO
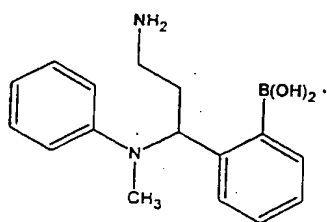
Target C
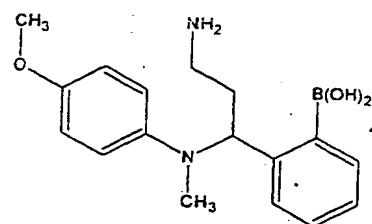
Target C-MeO
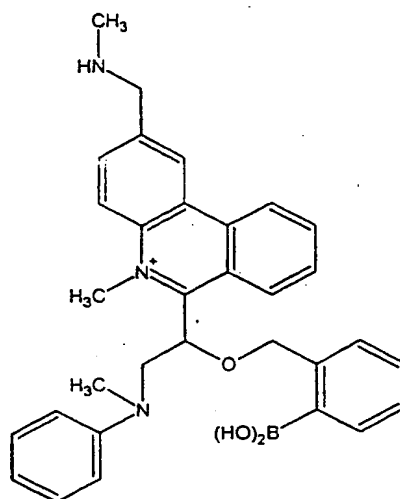
Target D
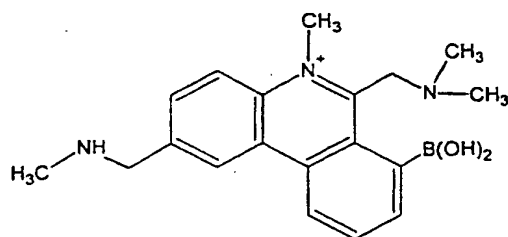
Target E
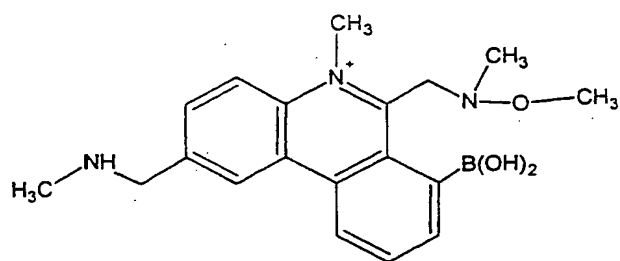
Target E-MeO
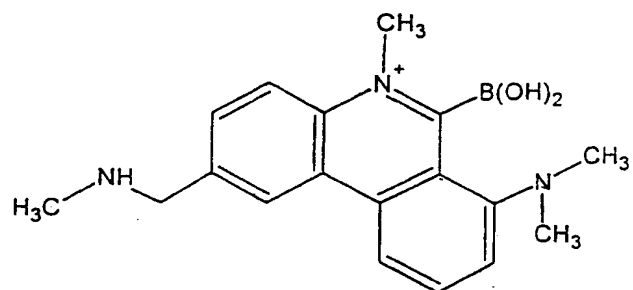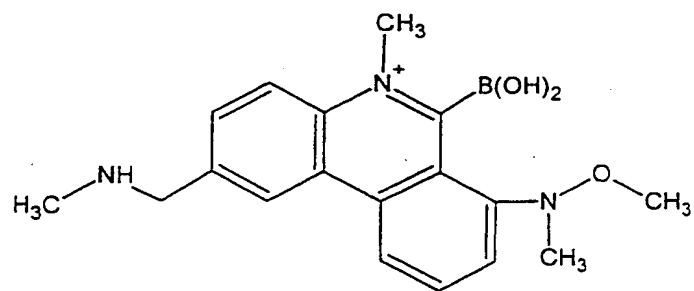
Target EE

Target EE-MeO
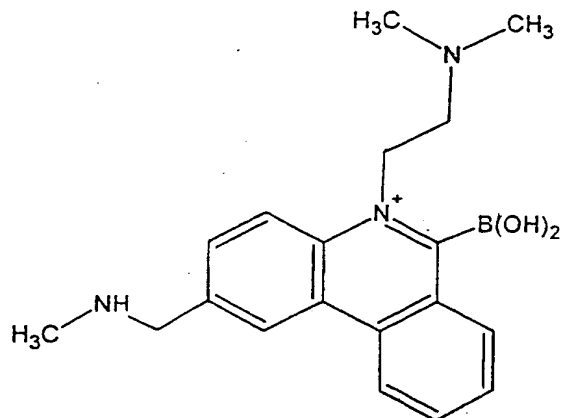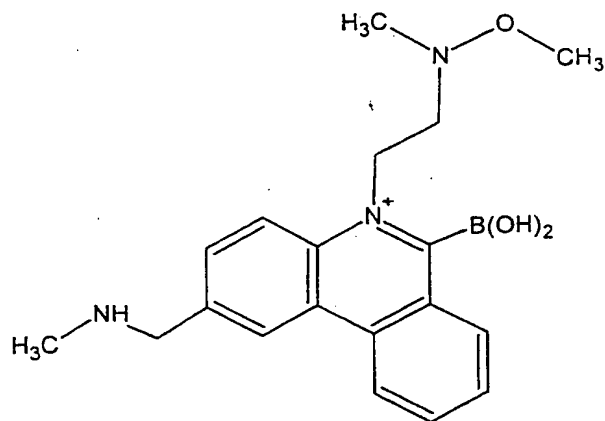
Target F

Target F-MeO
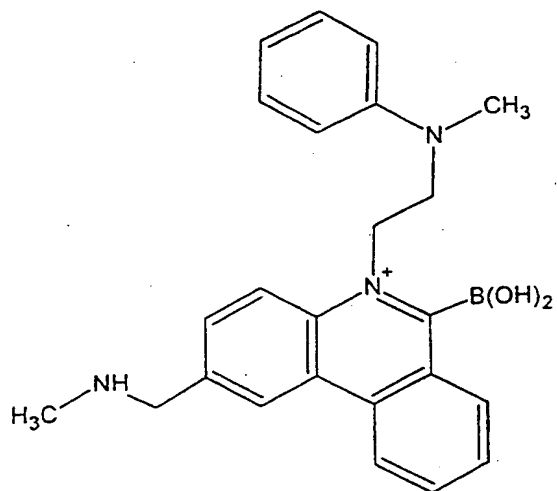
Target F-Phe
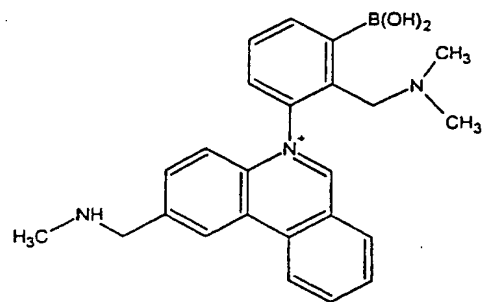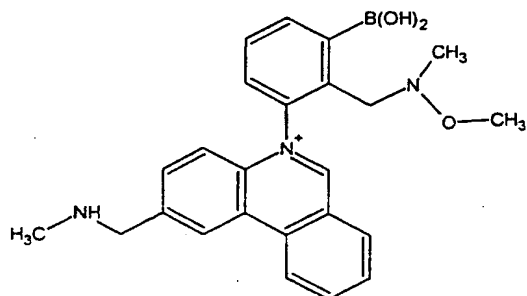
Target G

Target G-MeO
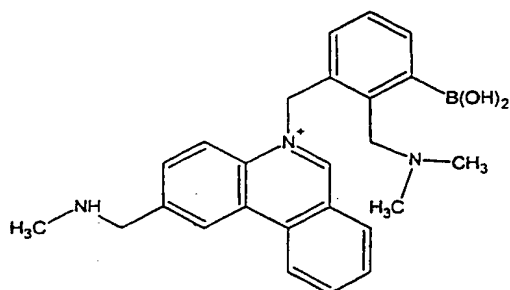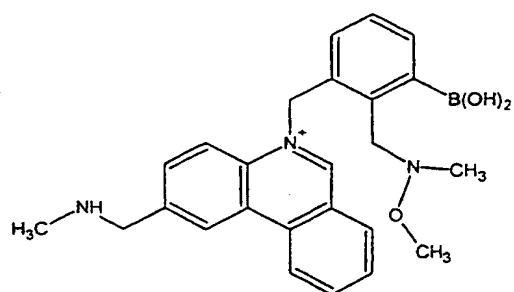
Target H

Target H-MeO
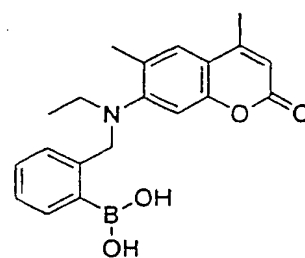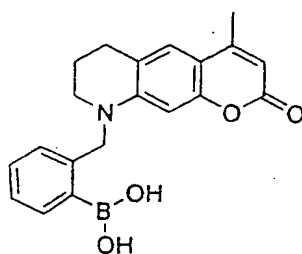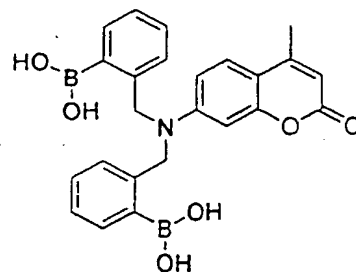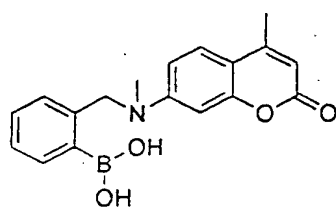
Examples of cis-diol analyte compounds other than glucose
include other sugars such as fructose and glycerol.
Catechols (o-dihydroxybenzenes) and catecholamines,
including hormones such as dopamine, epinephrine and
5   norepinephrine, contain adjacent (ortho) hydroxyls which
mimic cis-diols with respect to their reactivity with
boronate recognition elements.

The lanthanide metal ion M can be that of europium
(Eu^{3+}), samarium (Sm^{3+}), terbium (Tb^{3+}), dysprosium (Dy^{3+})
10   or neodymium (Nd^{3+}), and is preferably an ion of europium
(Eu^{3+}) or terbium (Tb^{3+}).

The ligand of the chelator Ch can also be an organic
ligand comprising any one or more of an alkane or alkene
group, preferably containing 1 to 10 carbon atoms, as
15   well as aromatic, carbocyclic or heterocyclic moieties,
including benzyl, napthyl, anthryl, phenanthryl or
tetracyl groups. The ligand can also comprise groups
such as $-CF_3$ and C_2F_5, as long as the ligand further
comprises a moiety to which an analyte-specific
20   recognition element R can be bound, if desired.
Additionally, any ligand of a chelate complex may be
inorganic instead of organic.

X represents the number of recognition elements R
bound to each of one or more chelators. X can be an
25   integer from 0 to 8, and in certain preferred embodiments
of the invention, X=0 to 4 or X=0 to 2. Additionally,
the number of recognition elements R bound to each of one
or more chelators may be the same or different, provided
that for one or more chelators, X>0. Y represents the
30   number of chelators complexed with M, and can be an
integer from 1 to 4. In certain preferred embodiments of
the invention, Y=1, Y=3 or Y=4.

In certain embodiments of the present invention, the
lanthanide metal chelate complex can comprise a mixture
35   of different chelators wherein one or more of the

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chelators do not contain an analyte-specific recognition
element R. The advantages of using such a mixed ligand
chelate, also known as a ternary ligand chelate, include
that some organic ligands, such as polyamino-

5   polycarboxylic acids, are more soluble in water than
other ligands, such as a β-diketone. Therefore, in at
least one embodiment of the invention, the lanthanide
metal chelate complex can comprise, first, one or more β-
diketones containing one or more analyte-specific

10   recognition elements, and second, one or more other
ligands such as a polyamino-polycarboxylic acid, which
promote the water solubility of the chelate complex.

In other embodiments of the present invention, one or
more chelators of the chelate complex can further

15   comprise an $-NH_2$ or $-OH$ group, or any other substituent by
which the chelate complex can attach covalently to a
linker or polymer such as a polylysine, or other solid
support.

In order to achieve energy transfer from the light

20   absorbing portion of the complex to the lanthanide metal
ion, the triplet state energy of the light absorbing
portion is preferably above about 230 kJ/mol. Preferred
light absorbing portions include phenanthridine (258
kJ/mol), psoralen (262 kJ/mol), phenoxazine (261 kJ/mol),

25   phenanthrene (258 kJ/mol), triphenylene (280 kJ/mol),
benzophenone (287 kJ/mol), carbazole (293 kJ/mol) and
coumarin (258 kJ/mol).

The fluorescence of the lanthanide metal chelate
complex of the present invention is modulated in an

30   analyte-specific manner by the binding of an analyte to
one or more chelators of the chelate complex through one
or more recognition elements R.

The fluorescent indicator molecules of the present
invention can be used for detecting a variety of

35   different possible chemical analytes which are reactive

-25-

with, and thus can be detected in a specific manner by,
an analyte-specific recognition element R. Preferred
analytes for detection using the present invention are
analytes such as glucose, fructose and other cis-diol

5   compounds. However, depending on the choice of the
recognition element, the indicator molecules of the
present invention are also useful to detect many other
analytes. For example, the following molecule has a
recognition element which makes it useful as a pH

10   indicator (see Lippitsch, *et al.*, *Sensors and Actuators* B
38-39 (1997) 96-102):

15

20
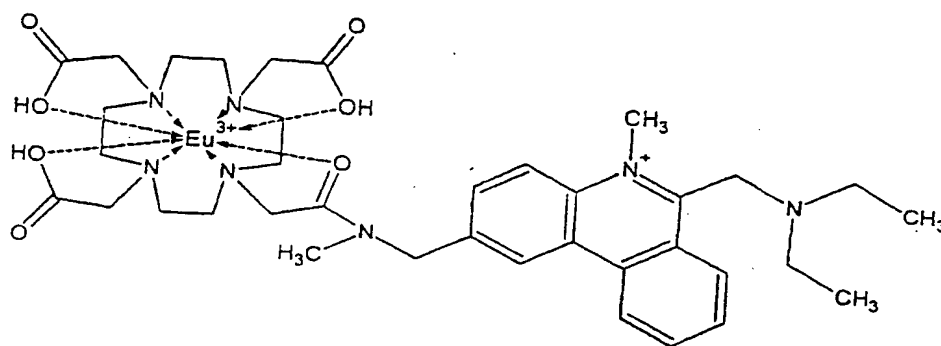
In addition, following is one of many possible compounds
which contains a recognition element which may be used to
bind a zinc analyte (see, e.g., Huston, et al., JACS
1988, 110, 4460):

5

10
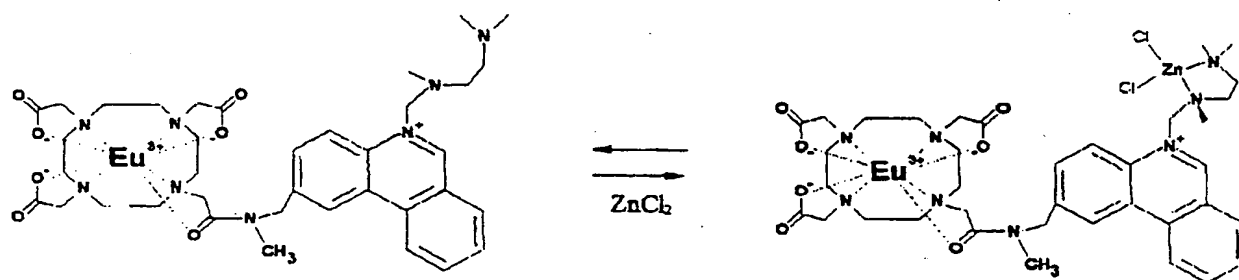
Zinc binding as shown will increase the fluorescence of
that indicator molecule, and others like it containing a
similar recognition element. Further, following is one
of many possible compounds which contains a recognition
element which may be used to detect a potassium analyte
(see, e.g., Sousa, et al., ACS Symposium Series 538,
1992, pp. 10-24):

5

10

15
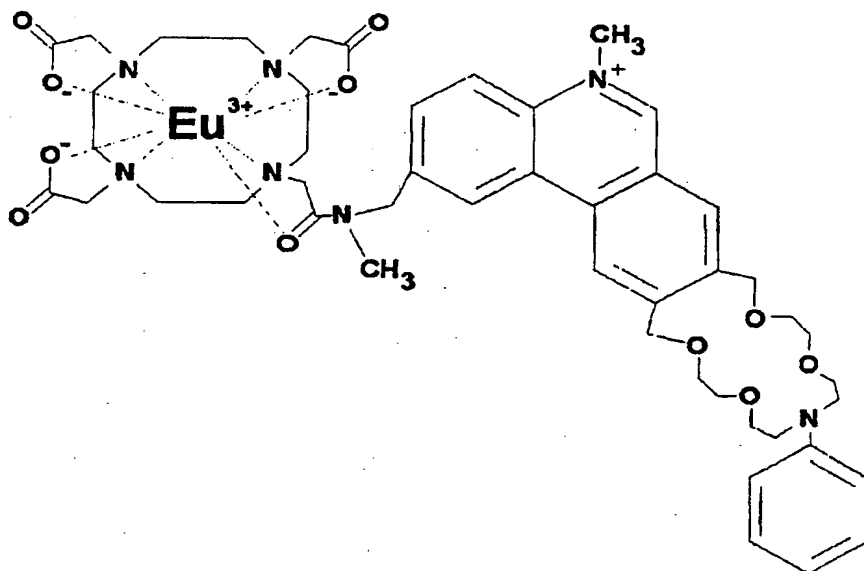
In that compound, the potassium ion is coordinated within
the crown ether moiety, causing a three-dimensional
configuration change such that the aniline portion of the
molecule is folded over onto the phenanthridine portion,
5   resulting in the quenching of fluorescence.

The chemical analytes detectable using the indicator
molecules of the present invention can exist in various
different solid, gaseous and liquid forms. Additionally,
analytes can be detected using the indicator molecules of
10   the present invention in various mediums, including both
liquid and gaseous mediums.

A number of possible uses exist for the fluorescent
compounds of the present invention, including uses as
indicator molecules in the fields of energy, medicine and
15   agriculture. For example, the fluorescent compounds can
be used as indicator molecules for detecting sub-levels
or supra-levels of glucose in blood or urine, thus
providing valuable information for diagnosing such
diseases as diabetes and adrenal insufficiency.
20   Medical/pharmaceutical production of glucose for human
therapeutic application requires monitoring and control.
Possible uses for the present invention in agriculture
include detecting levels of an analyte such as glucose in
soybeans and other agricultural products. Glucose must
25   be carefully monitored in critical harvest decisions for
such high value products as wine grapes. As glucose is
the most expensive carbon source and feedstock in
fermentation processes, glucose monitoring for optimum
reactor feed rate control is important in power alcohol
30   production. Reactor mixing and control of glucose
concentration also is critical to quality control during
production of soft drinks and fermented beverages, for
which production is consumed the largest amounts of
glucose and fermentable (cis-diol) sugars
35   internationally.

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Various detection techniques also are known in the
art that can make use of the fluorescent compounds of the
present invention. For example, the fluorescent
compounds of the invention can be used in fluorescent
5   sensing devices (e.g., U.S. Patent No. 5,517,313) or can
be bound to polymeric material such as test paper for
visual inspection. This latter technique would permit,
for example, glucose measurement in a manner analogous to
determining pH with a strip of litmus paper. The
10 fluorescent molecules described herein may also be
utilized as simple reagents with standard benchtop
analytical instrumentation such as spectrofluorometers or
clinical analyzers as made by Shimadzu, Hitachi, Jasco,
Beckman and others. These molecules would also provide
15 analyte specific chemical/optical signal transduction for
fiber optic-based sensors and analytical fluorometers as
made by Ocean Optics (Clearwater, Florida), or Oriel
Optics.

In a preferred embodiment of the present invention,
20 various possible chelators, one or more of which can be
complexed with a lanthanide metal ion, comprise an
organic ligand having attached thereto one or more
boronate groups as the analyte-specific recognition group
R, examples of which are shown below.

25

A.   ß-Diketones

The chelator Ch of the lanthanide metal chelate
complex of the present invention can be a ß-diketone-
30 based ligand, examples of which are provided below.
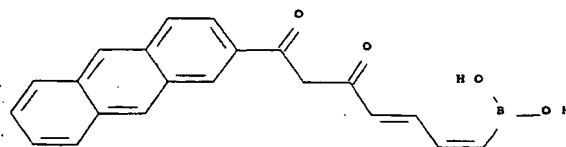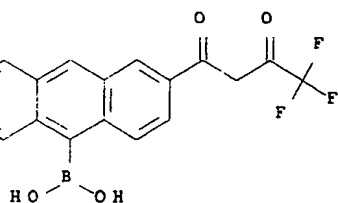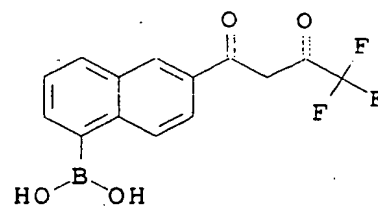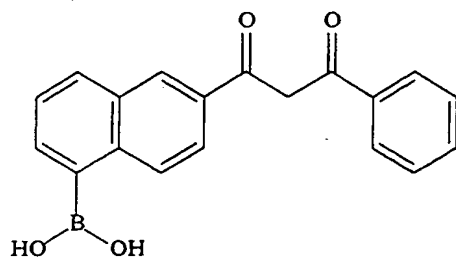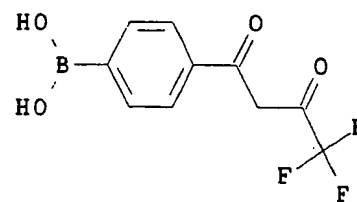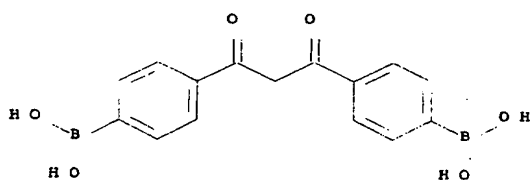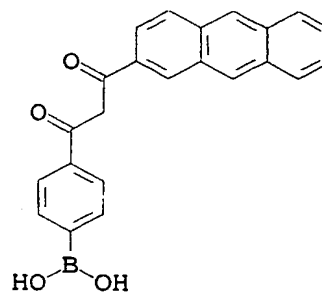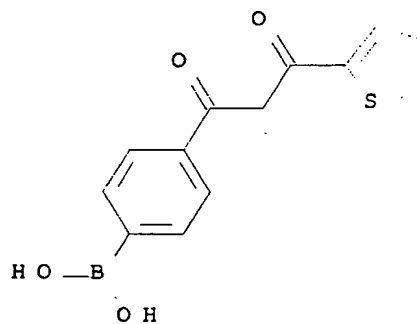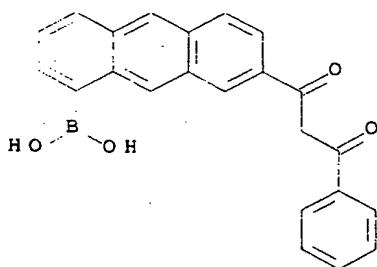
B. Macrobicyclic Cryptands (Cage-Type Ligands)

The chelator Ch in other embodiments of the present
invention can be a macrobicyclic cryptand (or cage-type
ligand), an example of which is shown below, having the
5   form:
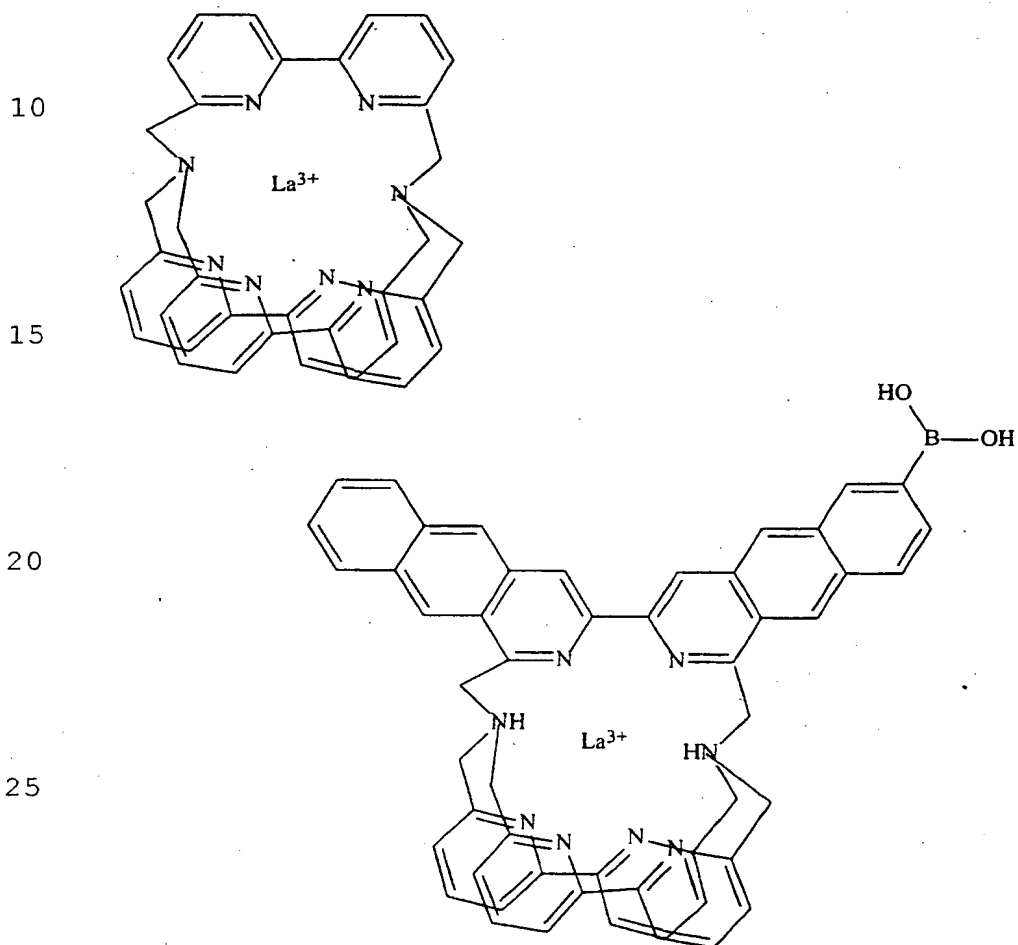
30   The chelator Ch in another embodiment of the present
invention can be a macrobicyclic cryptand having the
following structure.
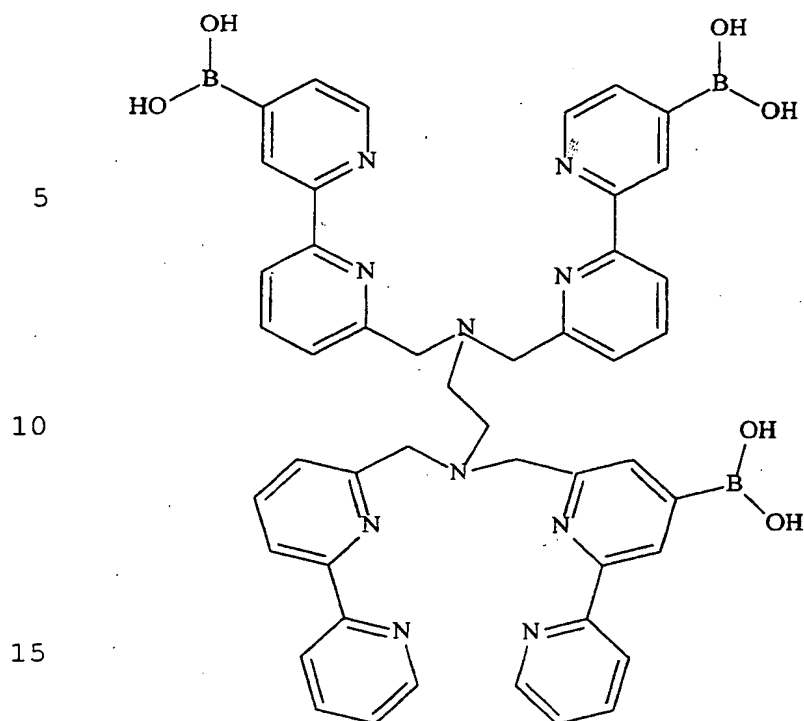
## C.   Nitrogen Heterocycles and Carboxylate Coordinate Ligands

Embodiments of the chelator Ch of the lanthanide metal chelate complexes of the present invention also include the following nitrogen heterocycles and carboxylate coordinate ligands.

-33-

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15
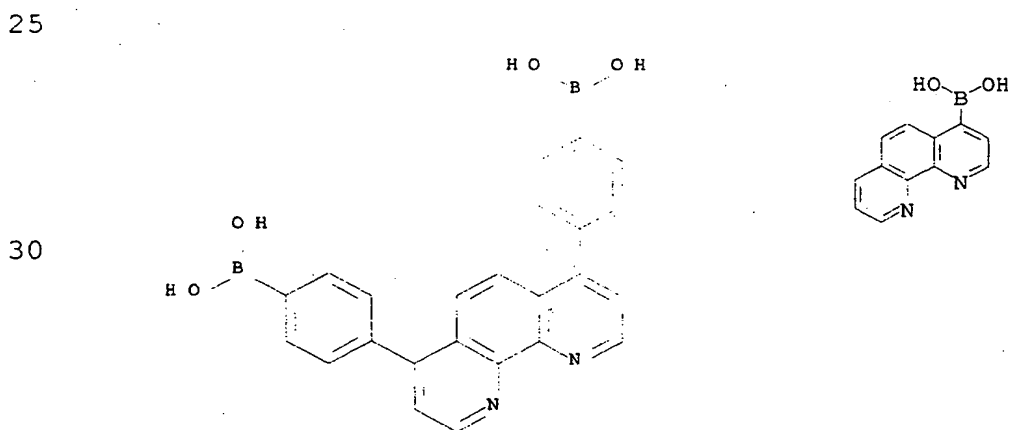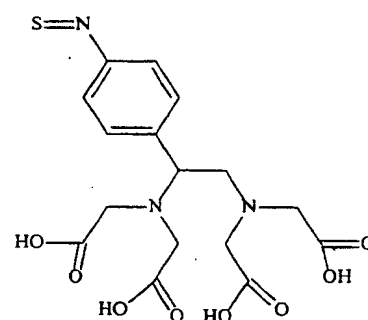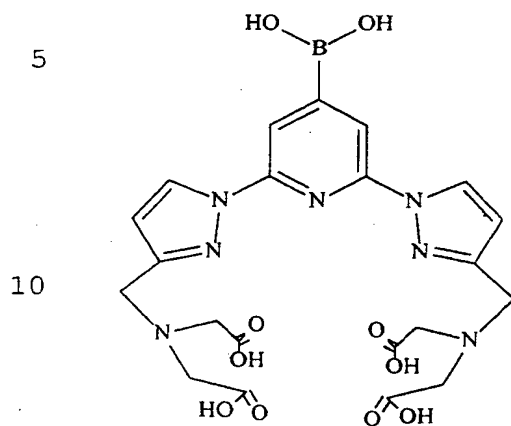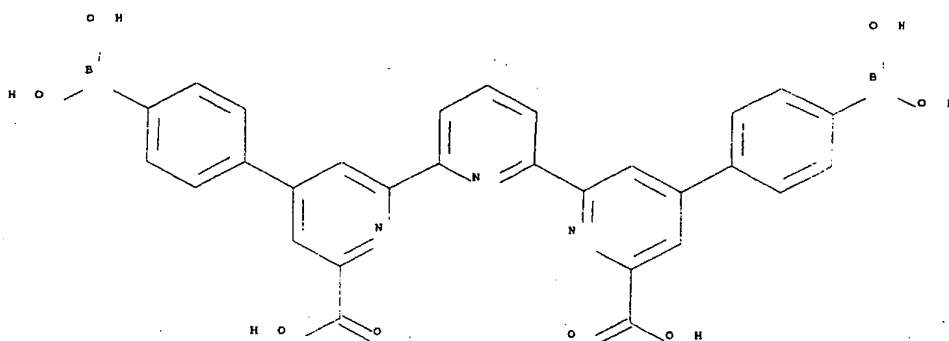
        Some of the most preferred fluorescent, boronate
recognition element-containing lanthanide chelates of the
present invention include the following europium chelates
(shown having only one ligand for purposes of clarity)
20   comprising the following structures:

Eu³⁺
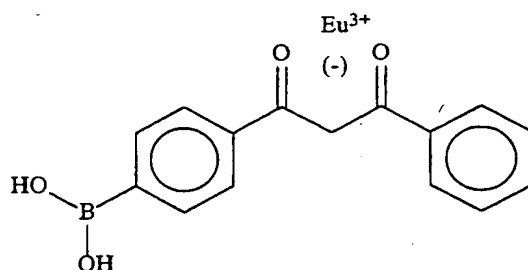
Europium-_p_-boronatedibenzoylmethane (II)

Eu³⁺
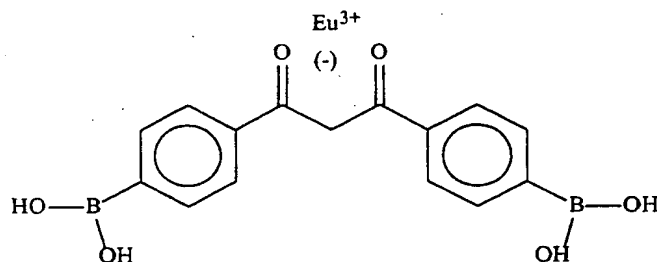
Europium Di-_p_-boronatedibenzoylmethane (III)

Eu³⁺
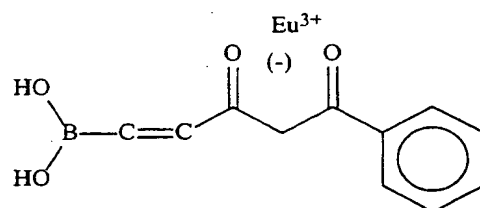
Europium Benzoylethylene methane-2-boronate (IV)

In a preferred embodiment of the present invention, a boronate-containing recognition element which is analyte-specific for glucose or other cis-diol compound was attached to a fluorescent europium (tetrakis) beta-naphthoyltrifluoroacetate (Eu-bNTA) chelate. The organic ligand portion of the chelate was known to form a shell around the lanthanide metal ion (e.g., europium), as drawn below:
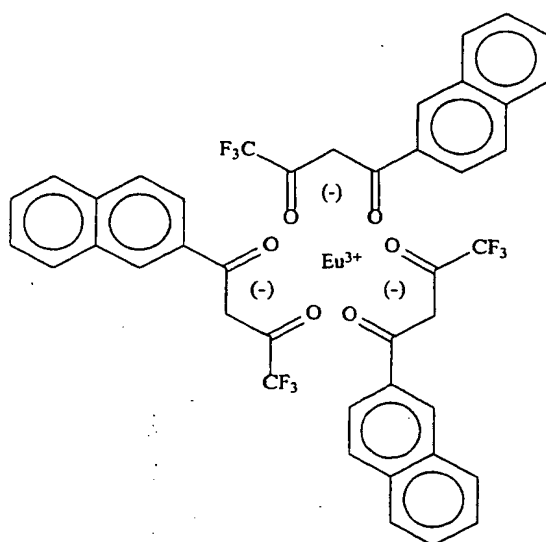
Additionally, that different solvents affect the
fluorescent decay time of the lanthanide metal ion (e.g.,
water quenches the fluorescence of the europium ion) also
was known. It was thus investigated whether modifying
the organic ligands of the outer shell with an analyte-
specific recognition element (e.g., a boronate-containing
recognition element) would perturb the decay time of the
europium ion to any notable extent. The following
europium chelate, europium (tetrakis)
naphthoyltrifluoromethane (Eu-bNTA) boronate, was
synthesized and tested.
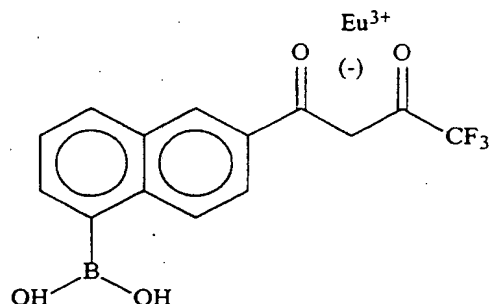
Eu-bNTA boronate was found to have an excitation
wavelength of about 340 nm and an emission wavelength

-36-

which was the same as for other europium chelates, about 613 nm.

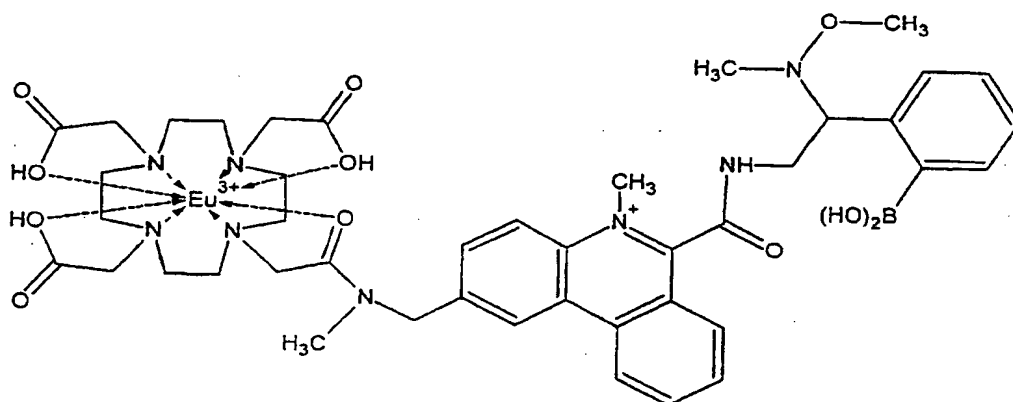Other preferred compounds for the detection of cis-diols including glucose include the following.
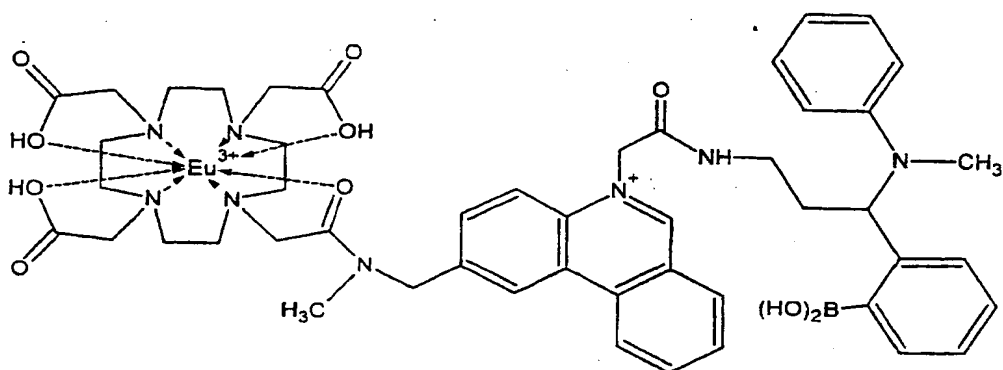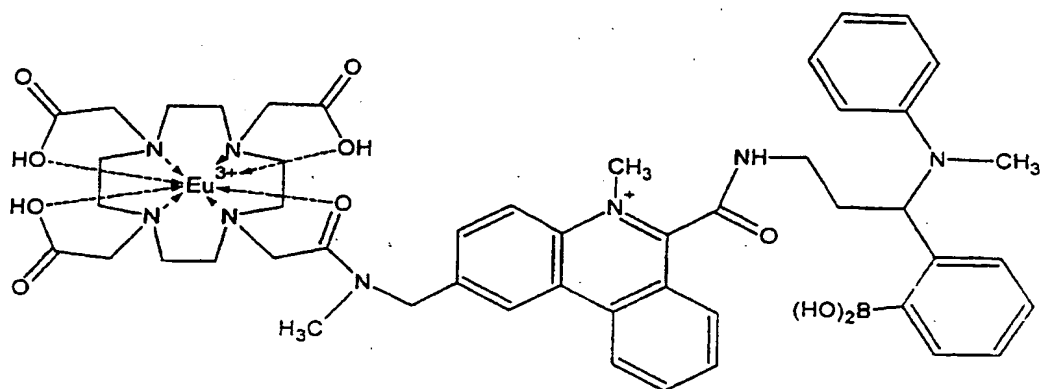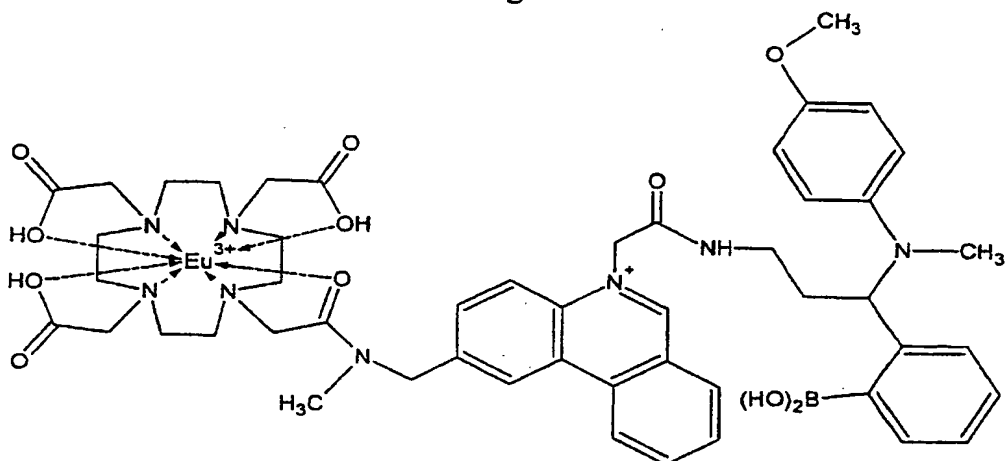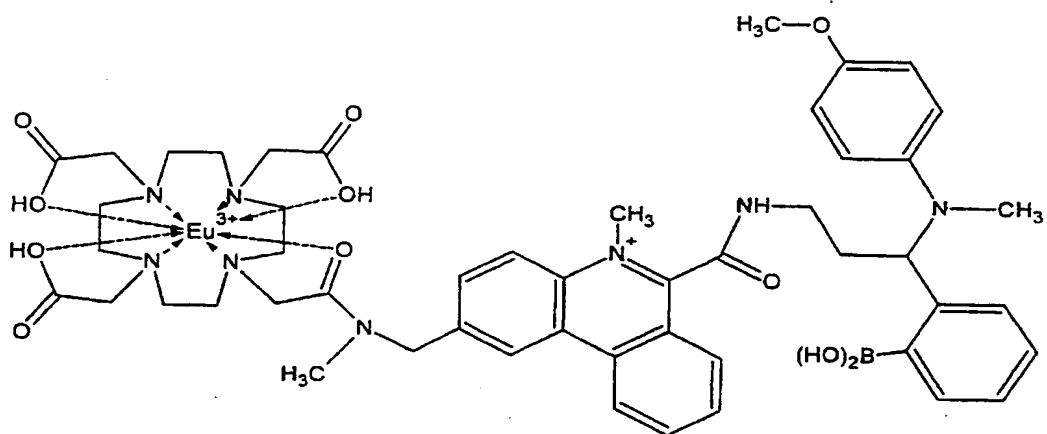
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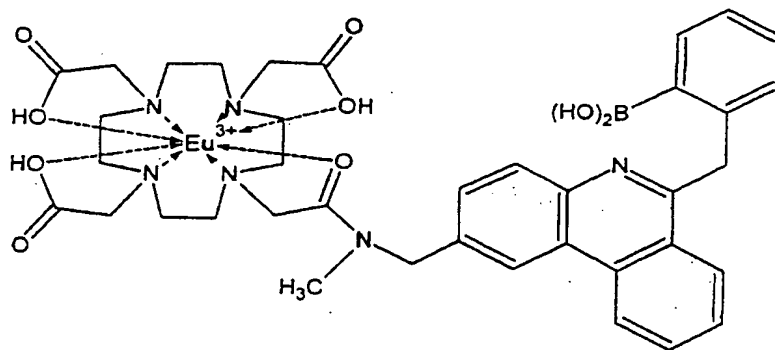
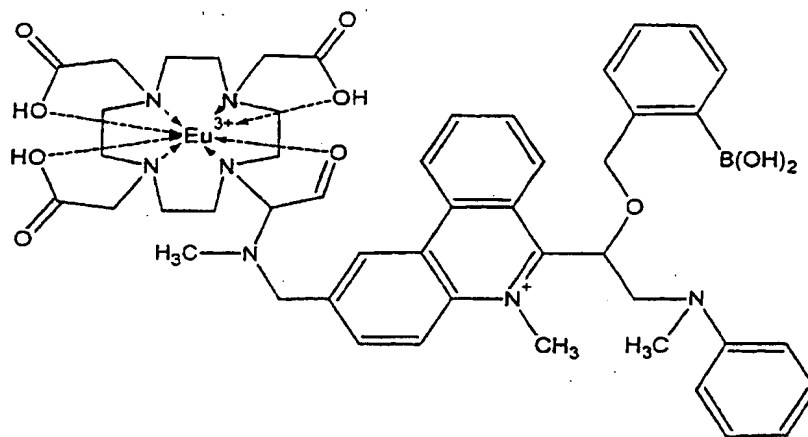Target A
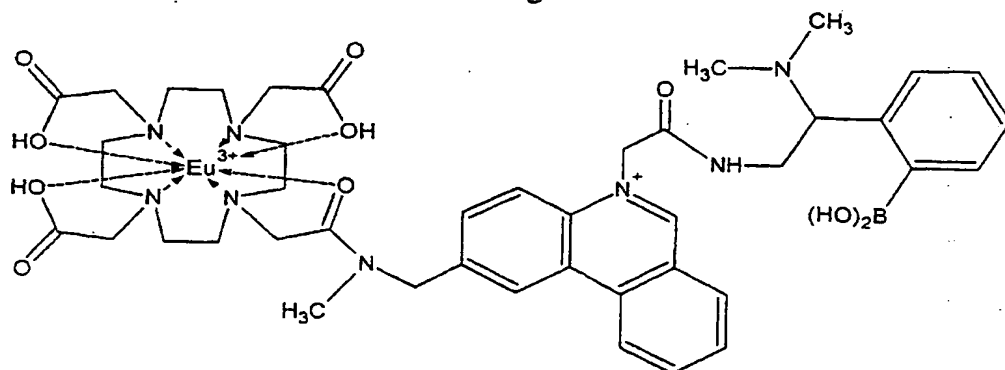
Target B-1
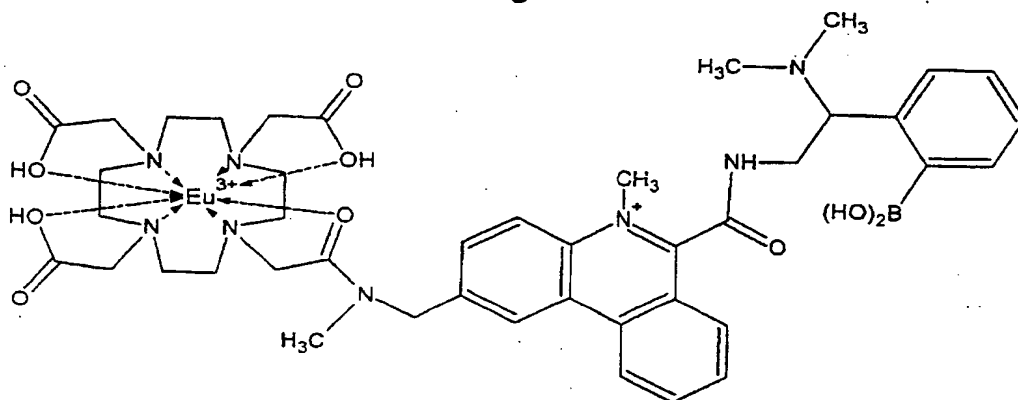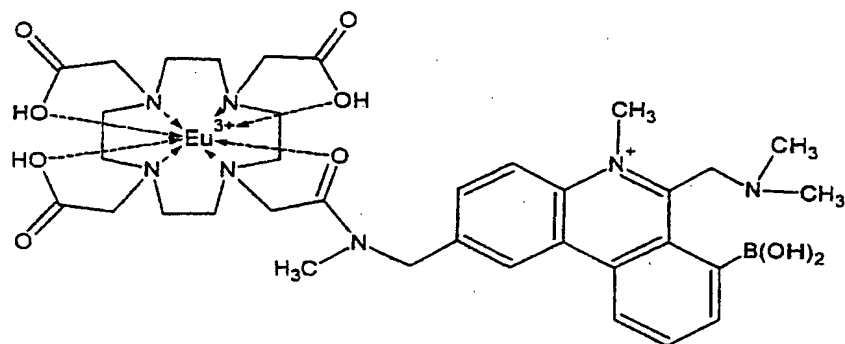
Target B-2
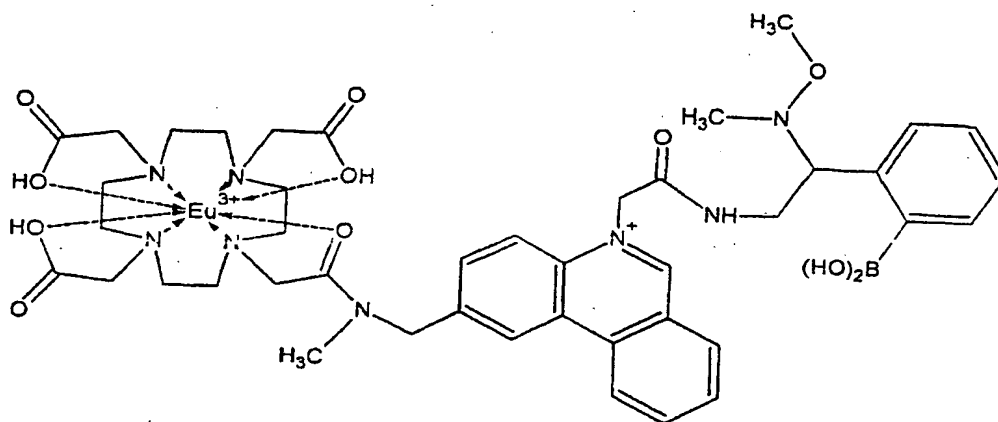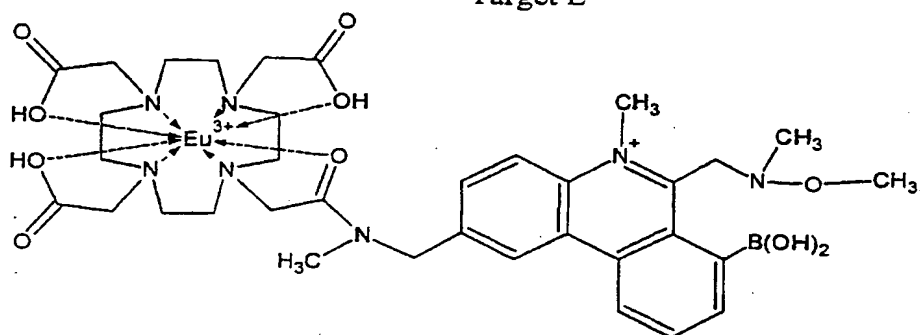
Target B-MeO-1

Target B-MeO-2

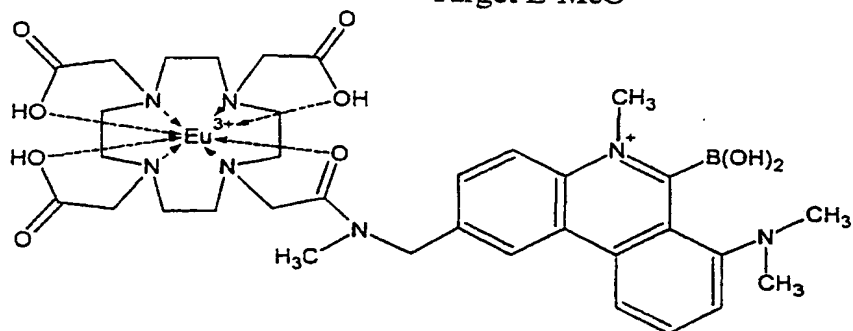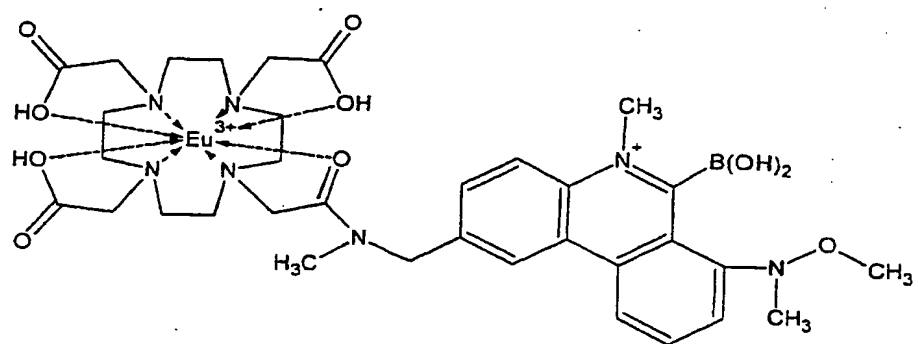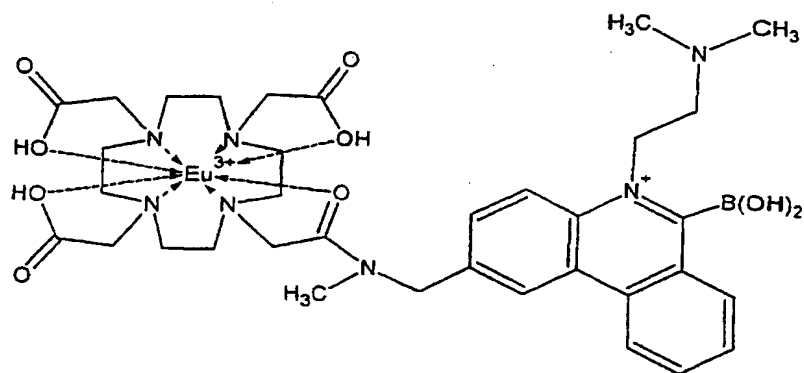
Target C-1

-39-

Target C-2
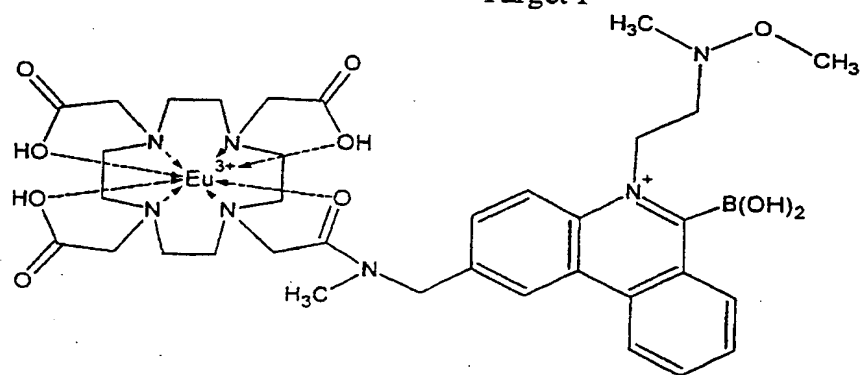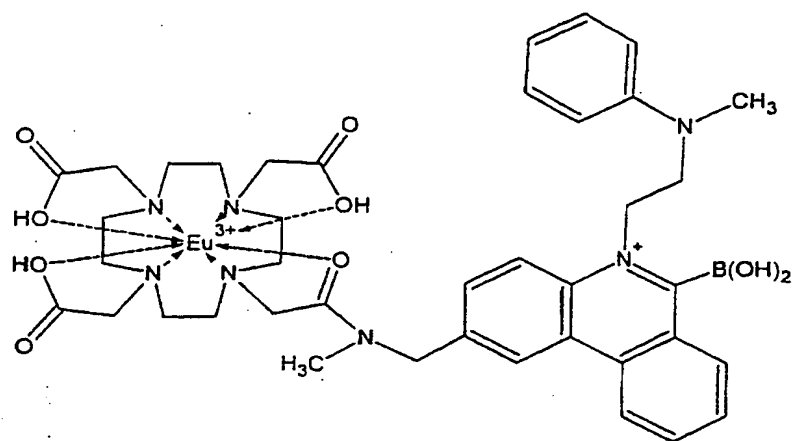
Target C-MeO-1

Target C-MeO-2
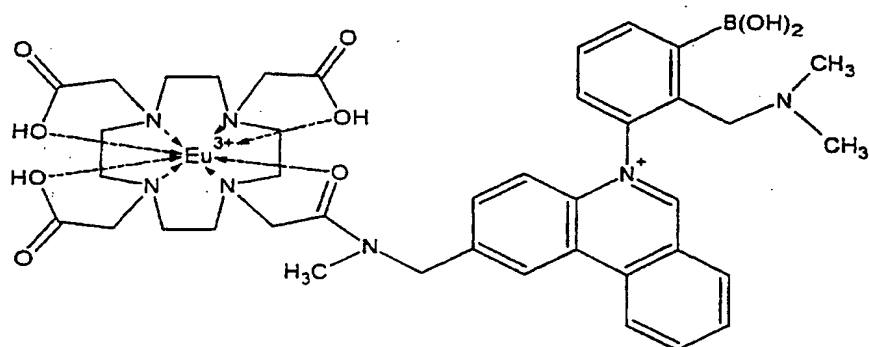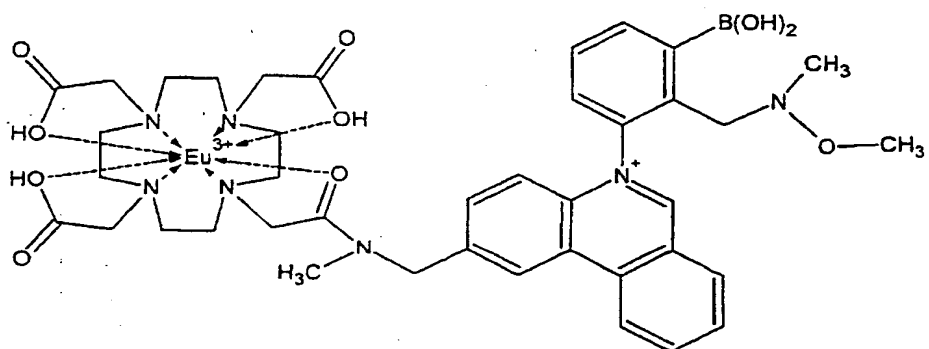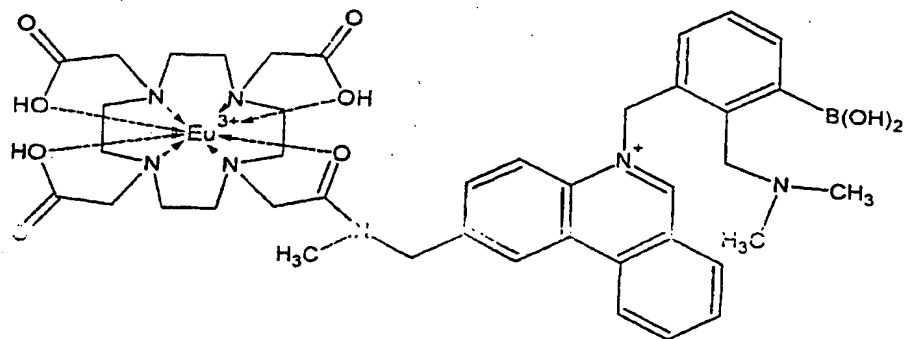
Target D

Target E

Target E-MeO

Target EE
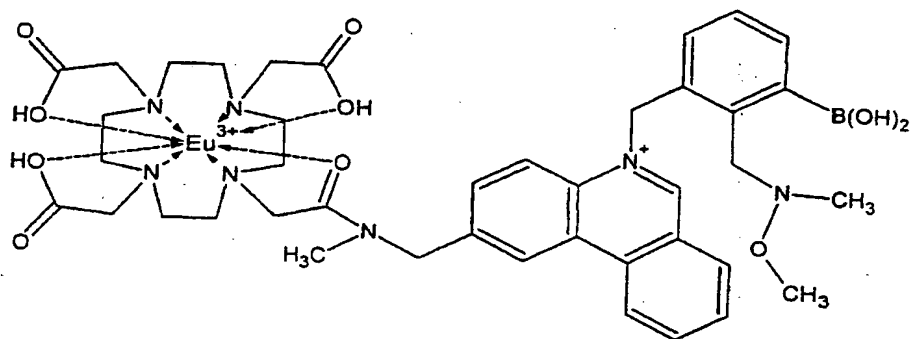
Target EE-MeO

Target F

Target F-MeO

Target F-Phe

Target G

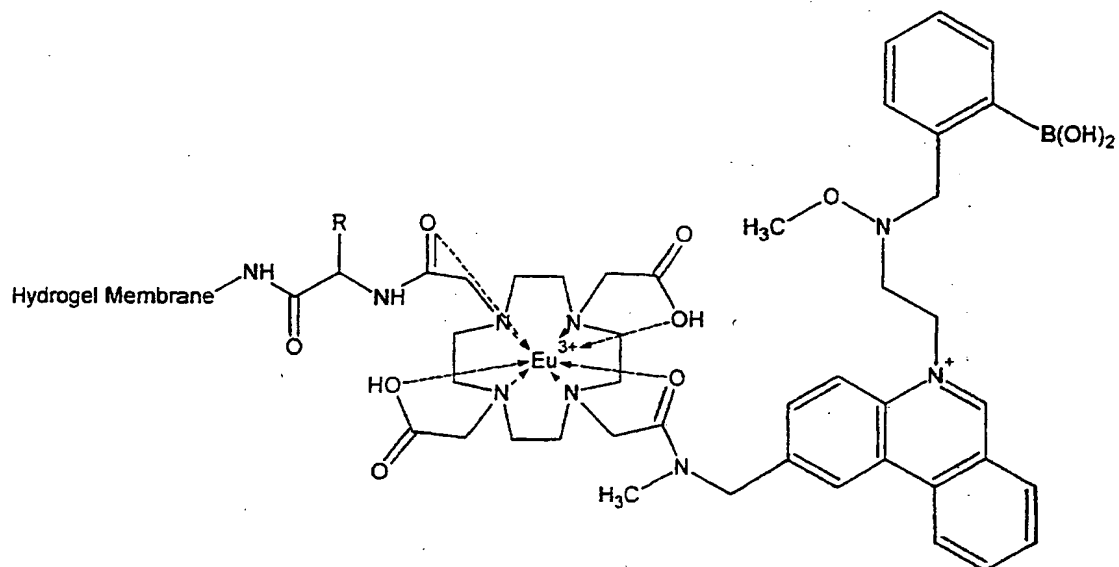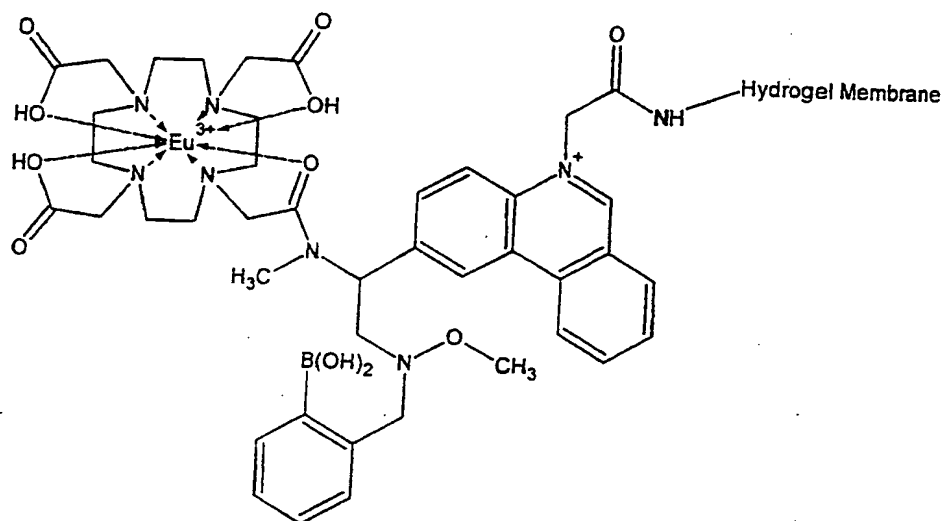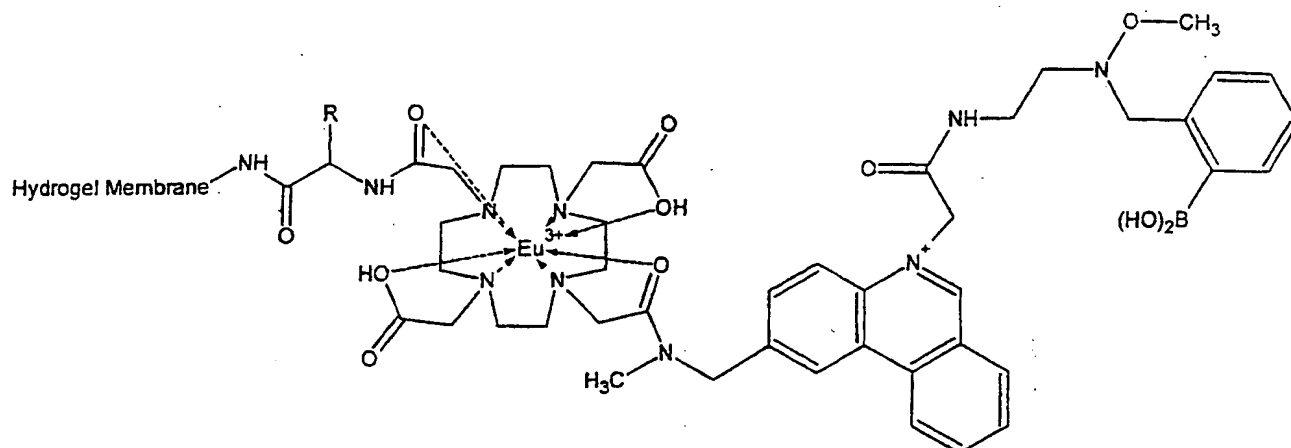
Target G-MeO

Target H

Target H-MeO

As shown in Figures 4-5, the advantages of the
present invention were demonstrated by a preferred
embodiment by measuring the effect of catechol (o-
dihydroxybenzene) on both the fluorescent intensity and
5   lifetime of Eu-bNTA in an ethanol solution. The changes
in fluorescent intensity detected and measured after
adding catechol to the ethanol solution in the presence
of Eu-bNTA are shown in Figure 5. The fluorescent
lifetime of Eu-bNTA without a boronate-containing
10   recognition element was 362 µs ±1 µs, while the
fluorescent lifetime of Eu-bNTA boronate decreased to 270
µs ±4 µs. After exposing the Eu-bNTA boronate to
catechol, the fluorescent lifetime of the molecule
further decreased to 209 µs ±15 µs.

15   The effect of catechol on the fluorescent intensity
and lifetime of Eu-bNTA boronate also was measured in
water and was shown to quench the fluorescence of the
europium ion of the chelate. Trioctylphosphine ("TOPO")
was then added to protect the coordination sites located
20   on the inner shell of the europium chelate complex from
the quenching effects of the water. The addition of TOPO
to the aqueous solution containing Eu-bNTA boronate is
depicted in Figure 3.

Eu-NTA boronate also was able to detect the presence
25   of glucose in methanol. Specifically, a glucose
titration with Eu-NTA boronate was performed in methanol.
Data obtained from the glucose titration are shown in
Figures 6-8. Figure 6 depicts a Eu-NTA boronate
titration against an increasing concentration of glucose.
30   Figure 7 depicts an expansion of the low range of the
data points shown in Figure 6. The results shown in
Figure 7 demonstrate that Eu-NTA boronate can detect the
presence of glucose at concentrations well below normal
physiological levels of approximately 4.7 mMol. Shown by
35   the data plot in Figure 7, differences in glucose

concentrations can be discriminated within the
physiological range of less than about 0.5 mMol.
Although Figure 7, because of the close proximity of data
points below .001 mMol glucose, does not show the lower
5   end sensitivity of Eu-bNTA boronate to changes in glucose
concentration, Figure 8 plots the low range of Figure 7
as the semi-log plot of glucose concentration versus I/I_o.
Figure 9 shows the results of titrating europium
dibenzoylmethane (Eu(DBM)) and Eu(boronated DBM)
10 separately with glucose and fructose in methanol.
Specifically, glucose and fructose concentrations were
varied as .0005, .005, .05, 1, 5, 10 and 20 mM. The data
plots for glucose B and fructose B in Figure 9 represent
glucose and fructose titrations of Eu(boronated DBM). It
15 is readily seen from the results in Figure 9 that the
fluorescent intensity of boronated europium
dibenzoylmethane increases significantly when exposed to
concentrations of glucose and fructose above about 0.01
mM, while the fluorescent intensity of the non-boronated
20 Eu(DBM) does not change to any notable extent upon the
addition of glucose and fructose. Without a specific
recognition element (e.g., a boronate), the lanthanide
metal chelate complex is not responsive to the presence
of glucose, fructose or other cis-diol compounds.
25 Accordingly, with an analyte-specific recognition
element, in this example a boronate group, the lanthanide
chelate complex of this invention is sensitive to the
presence of glucose, fructose and other cis-diol
compounds, and thus can be utilized to detect the
30 presence or concentration of such and other analytes.
Figures 10-13 further demonstrate the ability of
lanthanide metal chelate complexes in accordance with the
present invention to detect the presence or concentration
of glucose and/or fructose in a sample.

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As stated above, the fluorescent indicator molecules
of the present invention can be used in many different
types of fluorescent sensors. The fluorescent indicator
molecules can be used in the sensors to detect the
5   presence or concentration of an analyte such as glucose
or other cis-diol compound in a sample such as a liquid
sample, including a biological fluid, and more
specifically a human fluid. For example, fluorescent
indicator molecules in accordance with the present
10   invention can be dispersed in a polymer matrix which is
permeable to glucose or other cis-diol compound. The
presence or concentration of glucose or other cis-diol
compound in a medium such as a liquid medium can then be
determined by measuring the change in intensity or
15   lifetime of fluorescence emitted by the indicator
molecule after binding to glucose or other cis-diol
compound through one or more boronate-containing
recognition elements.

U.S. Patent 5,517,313, the disclosure of which is
20   incorporated herein by reference, describes a
fluorescence sensing device in which the fluorescent
indicator molecules of the present invention can be used
to determine the presence or concentration of an analyte
such as glucose or other cis-diol compound in a liquid
25   medium. The sensing device comprises a layered array of
a fluorescent indicator molecule-containing matrix
(hereafter "fluorescent matrix"), a high-pass filter and
a photodetector. In this device, a light source,
preferably a light-emitting diode ("LED"), is located at
30   least partially within the indicator material, such that
incident light from the light source causes the indicator
molecules to fluoresce. The high-pass filter allows
emitted light to reach the photodetector, while filtering
out scattered incident light from the light source.

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The fluorescence of the indicator molecules employed in the device described in U.S. Patent 5,517,313 is modulated, *e.g.*, attenuated or enhanced, by the local presence of an analyte such as glucose or other cis-diol

5   compound.

In the sensor described in U.S. Patent 5,517,313, the material which contains the indicator molecule is permeable to the analyte. Thus, the analyte can diffuse into the material from the surrounding test medium,

10   thereby effecting the fluorescence emitted by the indicator molecules. The light source, indicator molecule-containing material, high-pass filter and photodetector are configured such that at least a portion of the fluorescence emitted by the indicator molecules

15   impacts the photodetector, generating an electrical signal which is indicative of the concentration of the analyte (*e.g.*, glucose) in the surrounding medium.

In accordance with other possible embodiments for using the fluorescent indicator molecules of the present

20   invention, fluorescence sensing devices also are described in co-pending U.S. Patent Application Nos. 08/855,234, 08/855,235, and 08/855,236, all incorporated herein by reference.

The fluorescent indicator molecules of the present

25   invention can be prepared by persons skilled in the art without an undue amount of experimentation using readily known reaction mechanisms and reagents, including reaction mechanisms which are consistent with the general procedures described below.

30

Preparation of Boronated
Europium Tetrakis ß-Diketone Complexes

1.   Naphthalene-1-boronic acid, as available from Frontier Scientific (Logan, Utah), is dissolved in

35   toluene.

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2. The boronic acid must first be protected (capped) by
reacting it with 2,2-dimethyl-1,3-propanediol (Aldrich
Chemical Company) while azeotropically removing water by
using a Dean-Stark trap to provide 2,2-dimethylpropane-
5   1,3-diyl 1-naphthylboronate, as shown below:
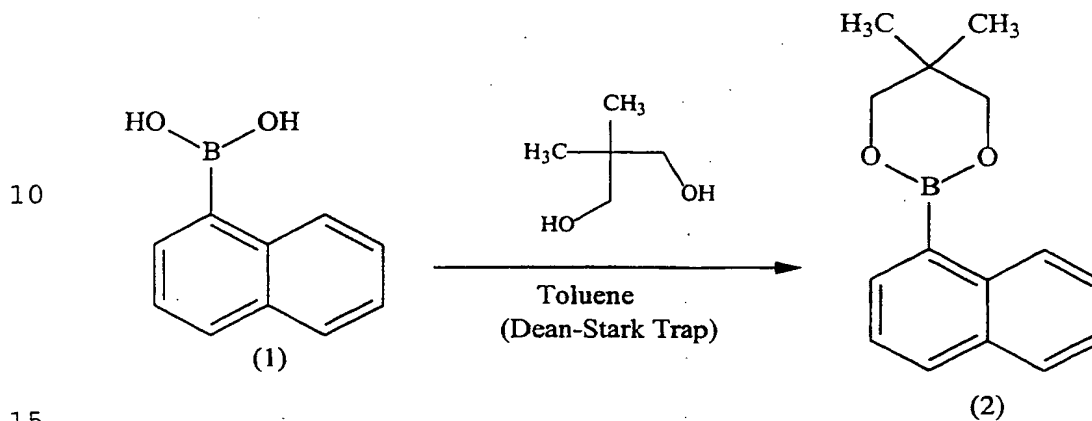
3. The capped boronic acid can then be acylated by
Friedel-Crafts acetylation by reacting the boronic acid
with acetic anhydride and aluminum trichloride in
anhydrous carbon disulfide to produce 2,2-
20   dimethylpropane-1,3-diyl 5-acetyl-1-naphthylboronate. An
approximately 70% yield of the reaction product, which
appears as a viscous liquid, will typically result, as
shown below:
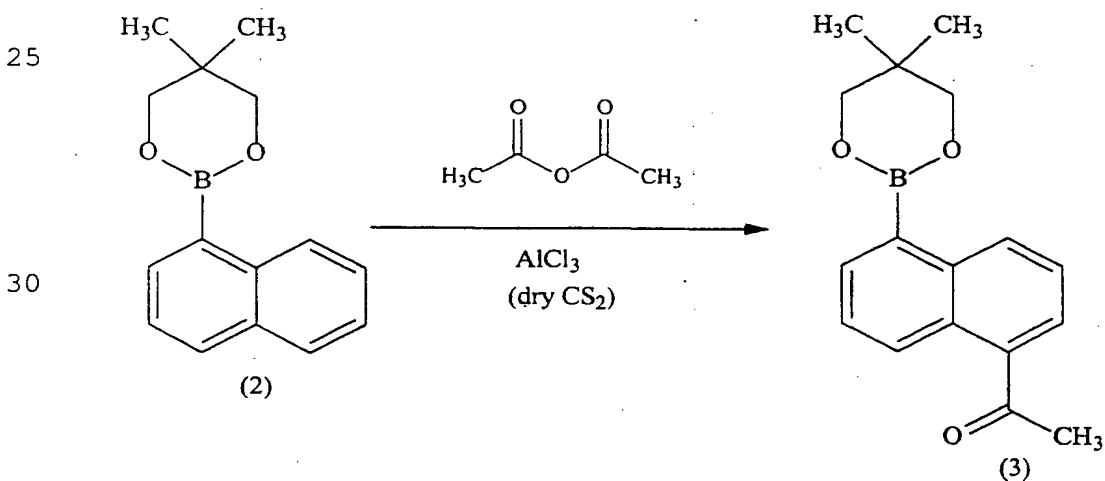
4.   The β-diketone can then be formed and the boronate can be decapped by a Claisen condensation between (3) and ethyl trifluoroacetate (Aldrich) using sodium methoxide (in dry ether) as the condensing agent, as shown below:

(3)

CH₃ONa
(dry ether)

(4)
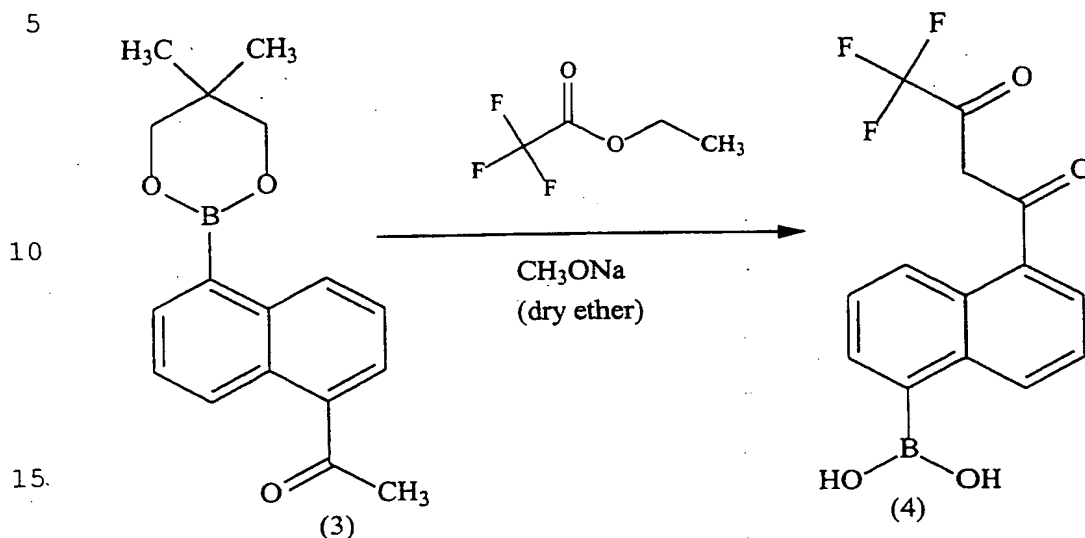
5.   The intermediate product (4), 5-naphthoyl-trifluoroacetoneboronic acid, can then be purified by preparative silica gel TLC while eluting with methylene chloride.   In at least one preparation, the third band elution (Rf=0.70-0.85) was recovered from the plate and analyzed by proton NMR at 400 MHZ. The NMR spectrum. exhibited a pattern which was characteristic of the enol form of the β-diketone, specifically showing peaks at sigma 6.69 (singlet) and at sigma 15.28 (broad singlet).

6.   The final europium tetrakis indicator complex containing a boronate group as the analyte-specific recognition element is produced by reacting the β-diketone (4), with europium trichloride hexahydrate (Aldrich) and piperidine in absolute ethanol, as shown below:

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(4)

EuCl₃6H₂O
(absolute ethanol)

(5)
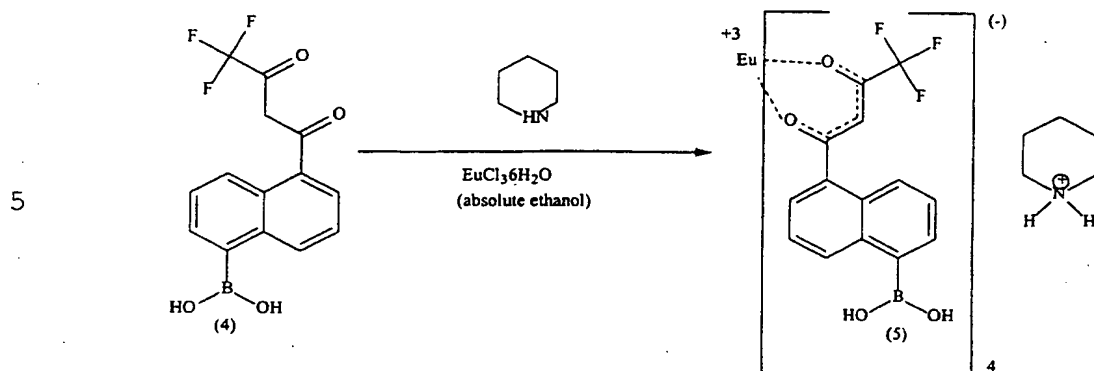
The solution is then heated to 70°C for three hours. After heating, the resultant solution shows a characteristic orange-red emission upon irradiation with a handheld, long-wave UV source. Additionally, a fluorescence scan with a Shimadzu fluorometer shows a peak excitation wavelength of 340 nanometers and a europium complex characteristic peak emission wavelength of 613 nanometers.
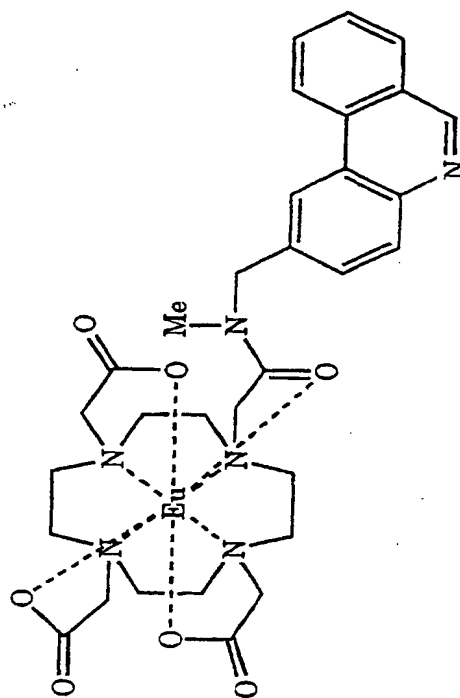
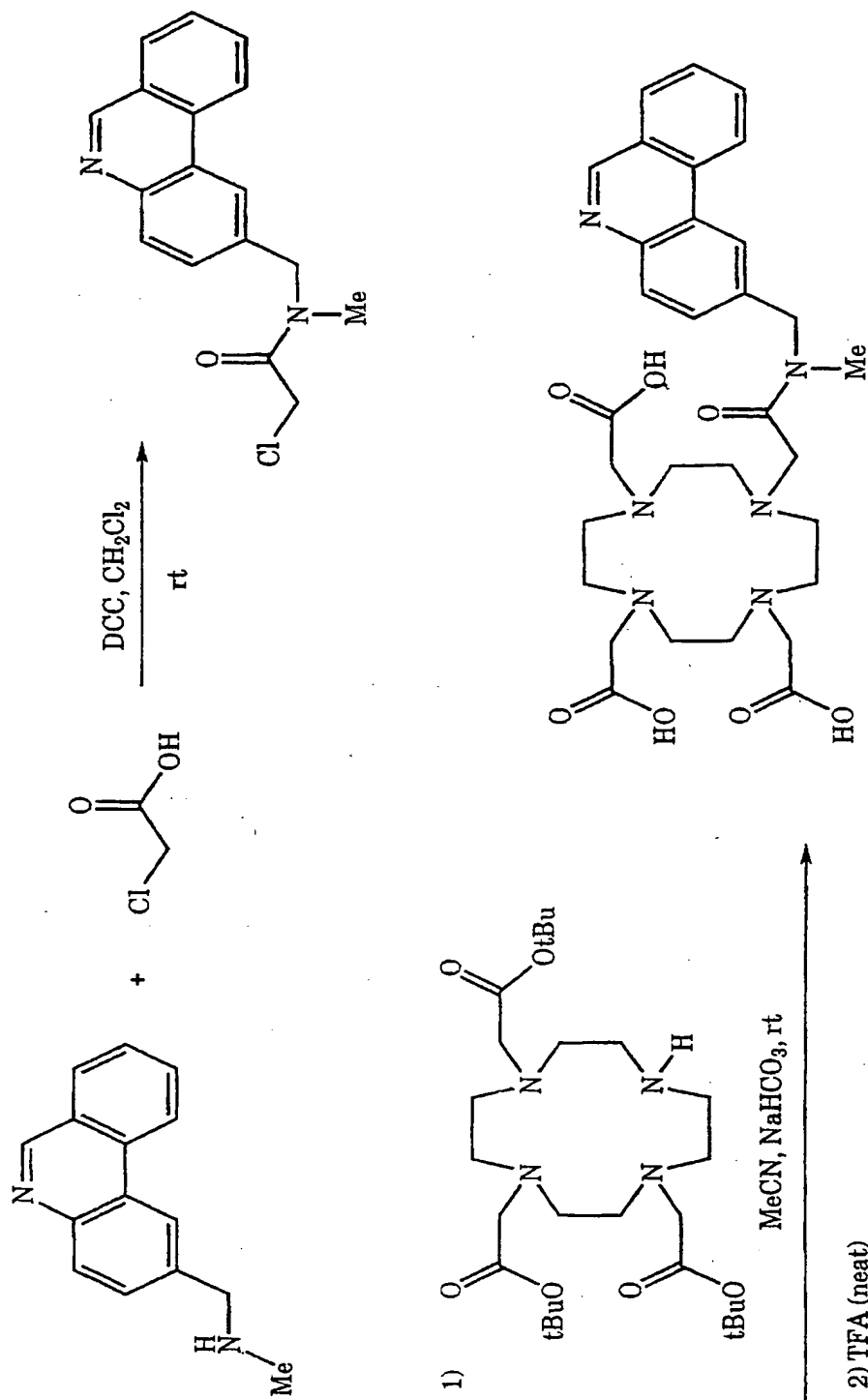Other synthetic schemes for preparing compounds useful in the present invention are depicted below.
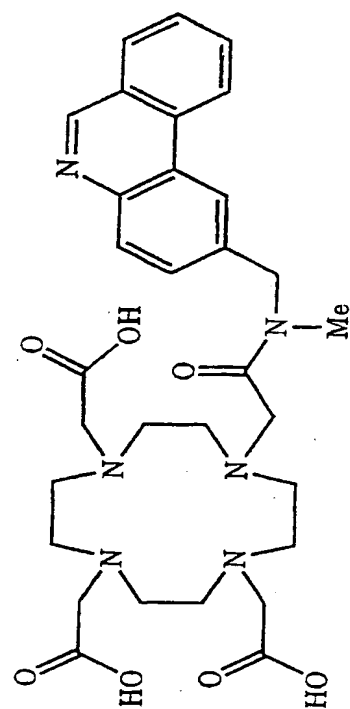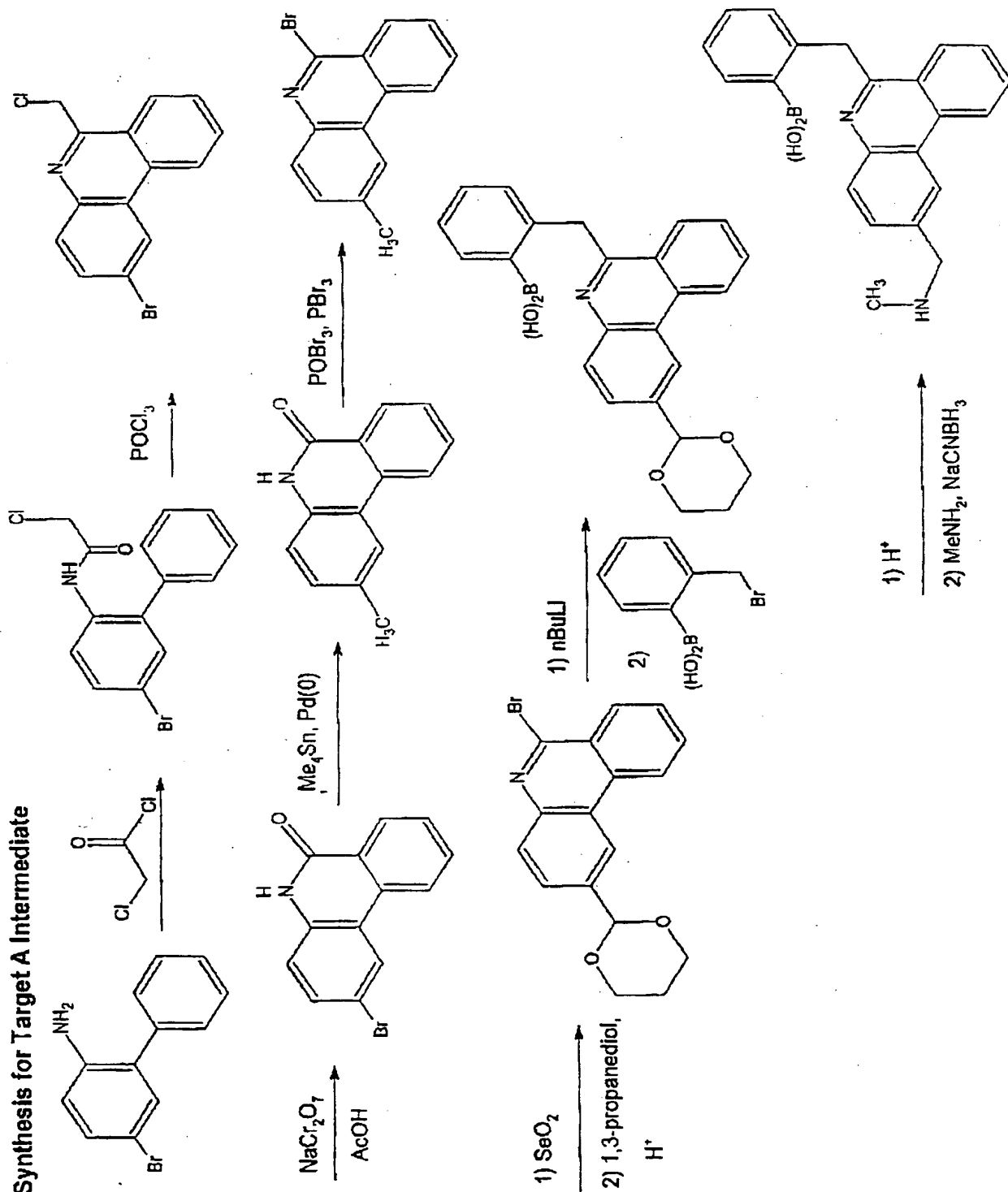
1) EuCl₃, pH 5-6

2) purify by alumina chromatography

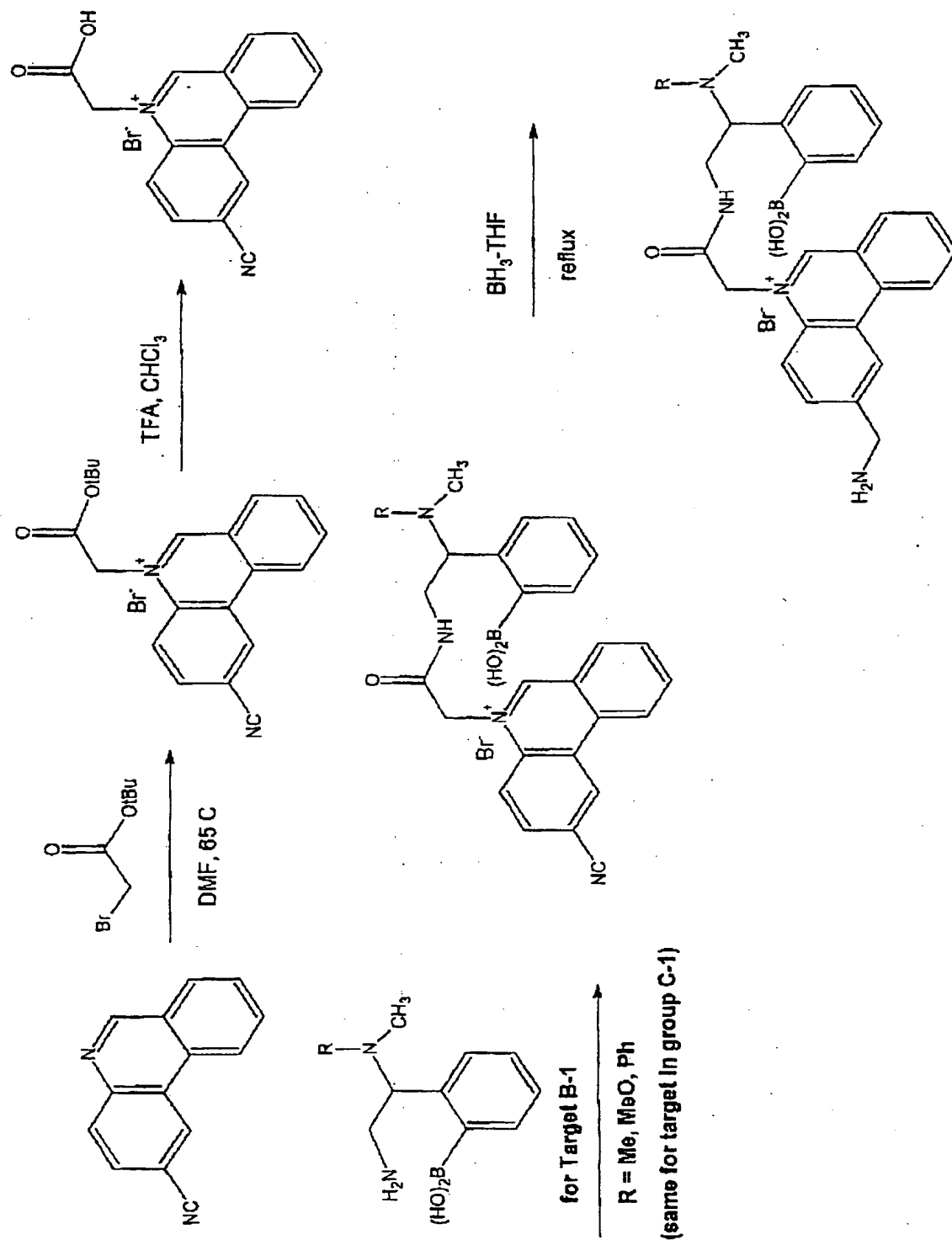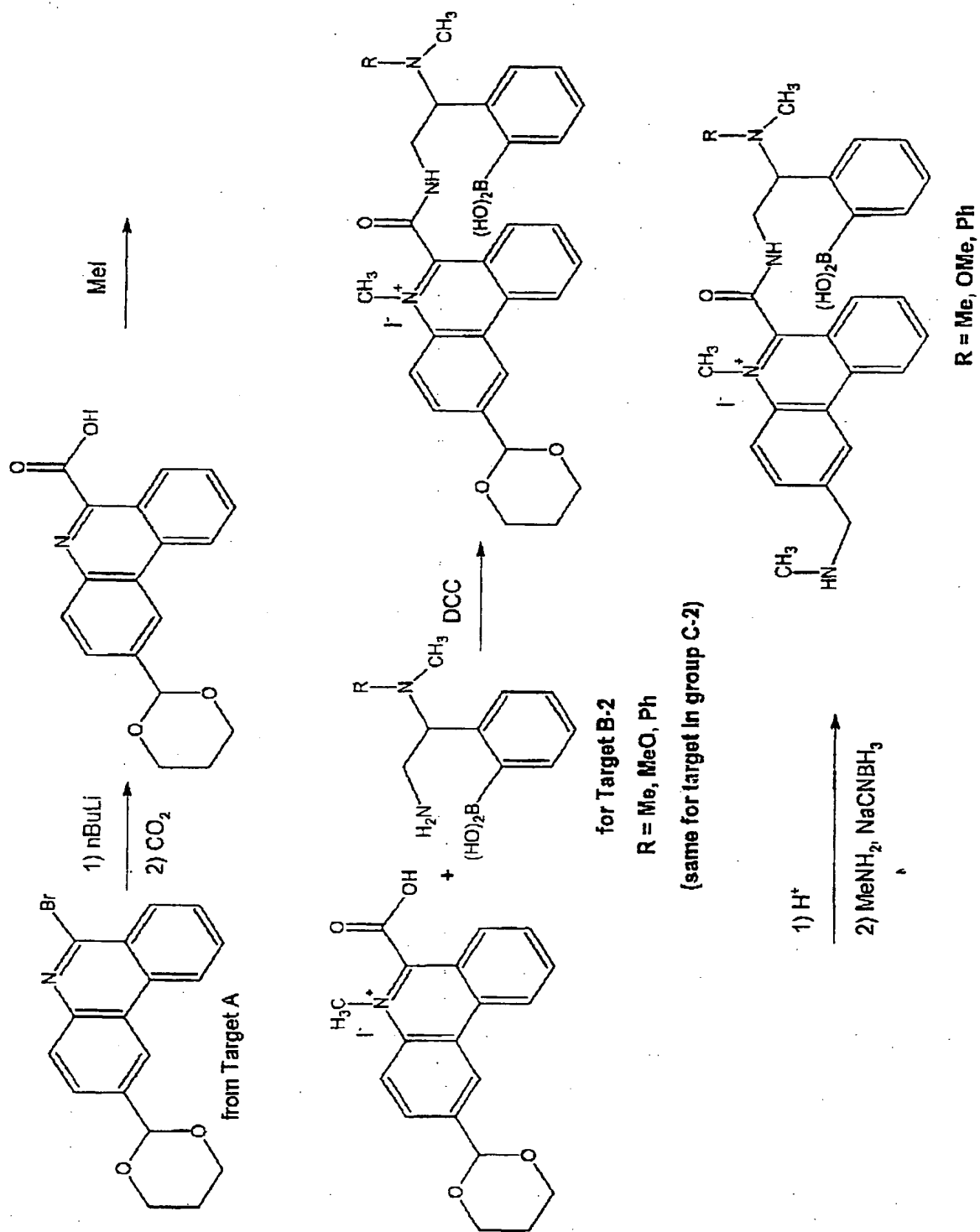**Synthesis for Target A Intermediate**

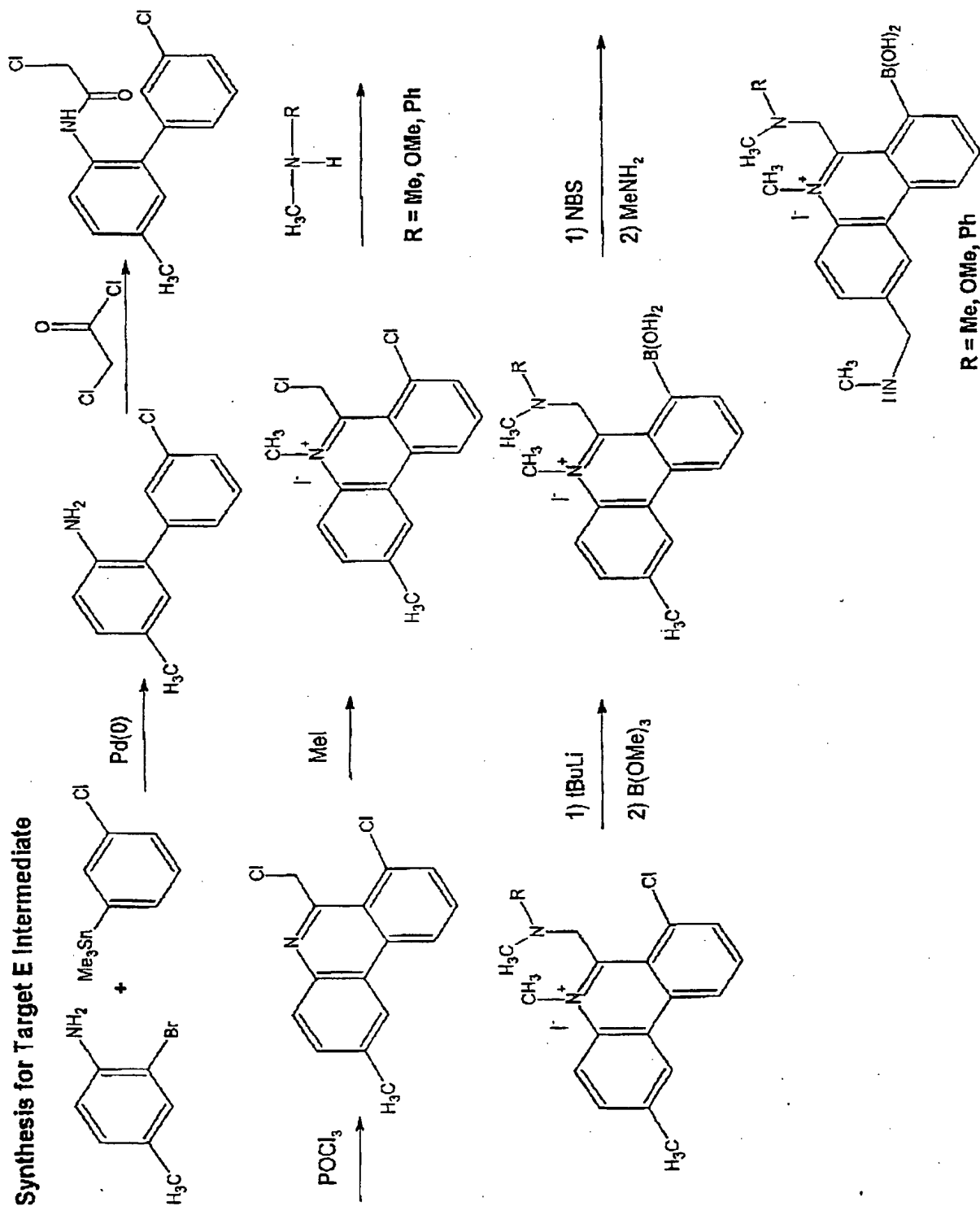Synthesis for Target B-1, C-1 Intermediates

Synthesis for Target B-2, C-2 Intermediates

Synthesis for Target E Intermediate

Synthesis for Target EEE Intermediate
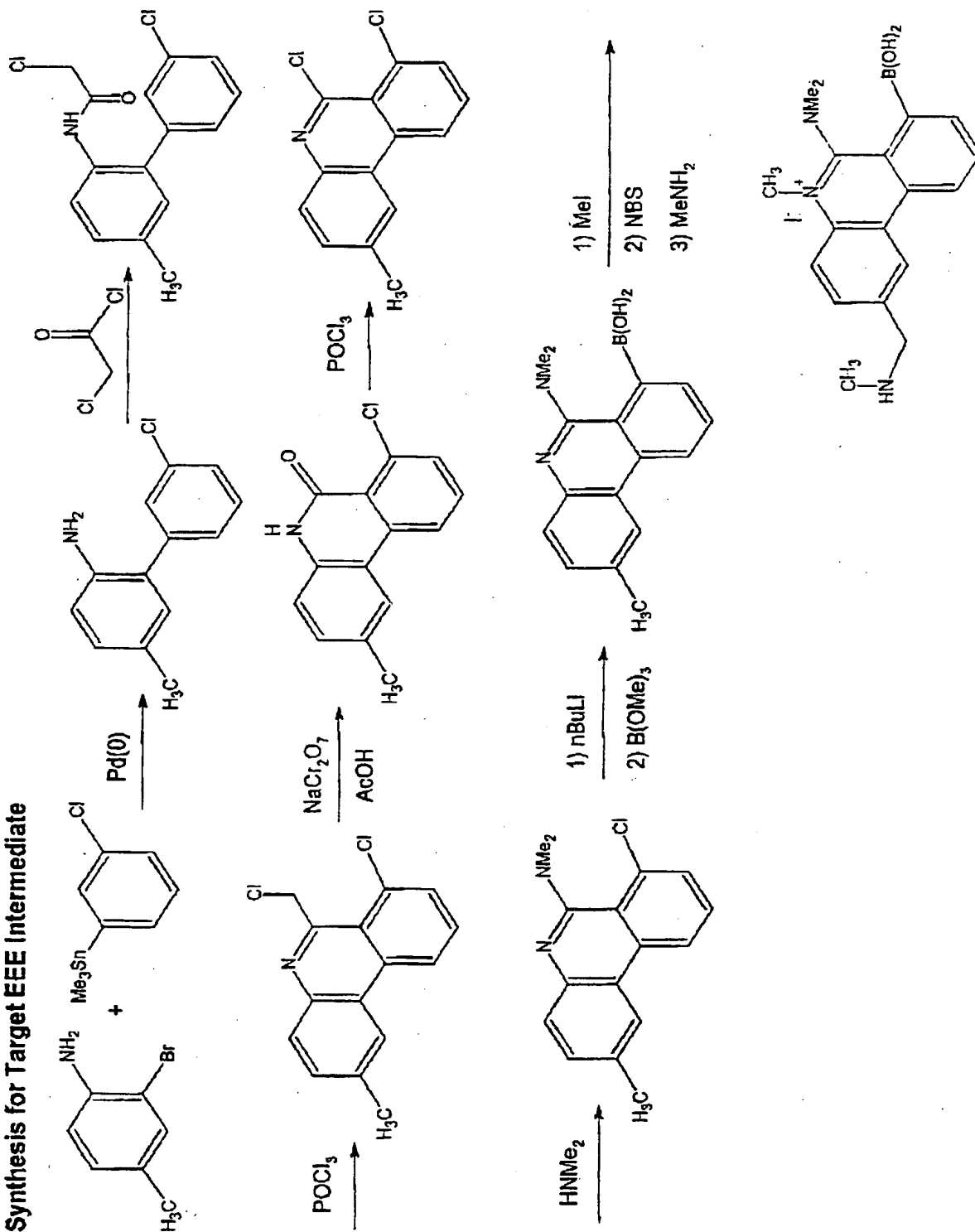
Synthesis for Target F Intermediate

from Target A

1) nBuLi
2) B(OMe)₃

Br⁻OH
DMF, 80 C

SOCl₂

$H_3C-\overset{\displaystyle H}{\underset{\displaystyle |}{N}}-R$

R = Me, OMe, Ph

1) H⁺
2) MeNH₂, NaCNBH₃

Target F

R = Me, OMe, Ph

Target H - Synthesis

1) DIBAL

2) 1,3-propanediol, benzene, cat TsOH

1) Mg⁰, ether

2) B(OMe)₃

Aldrich Chemical Co.

St. Louis, MO

NBS, CCl₄

1) DMF

2) H⁺

1) HNMe₂, NaCNBH₃, EtOH

2) BH₃
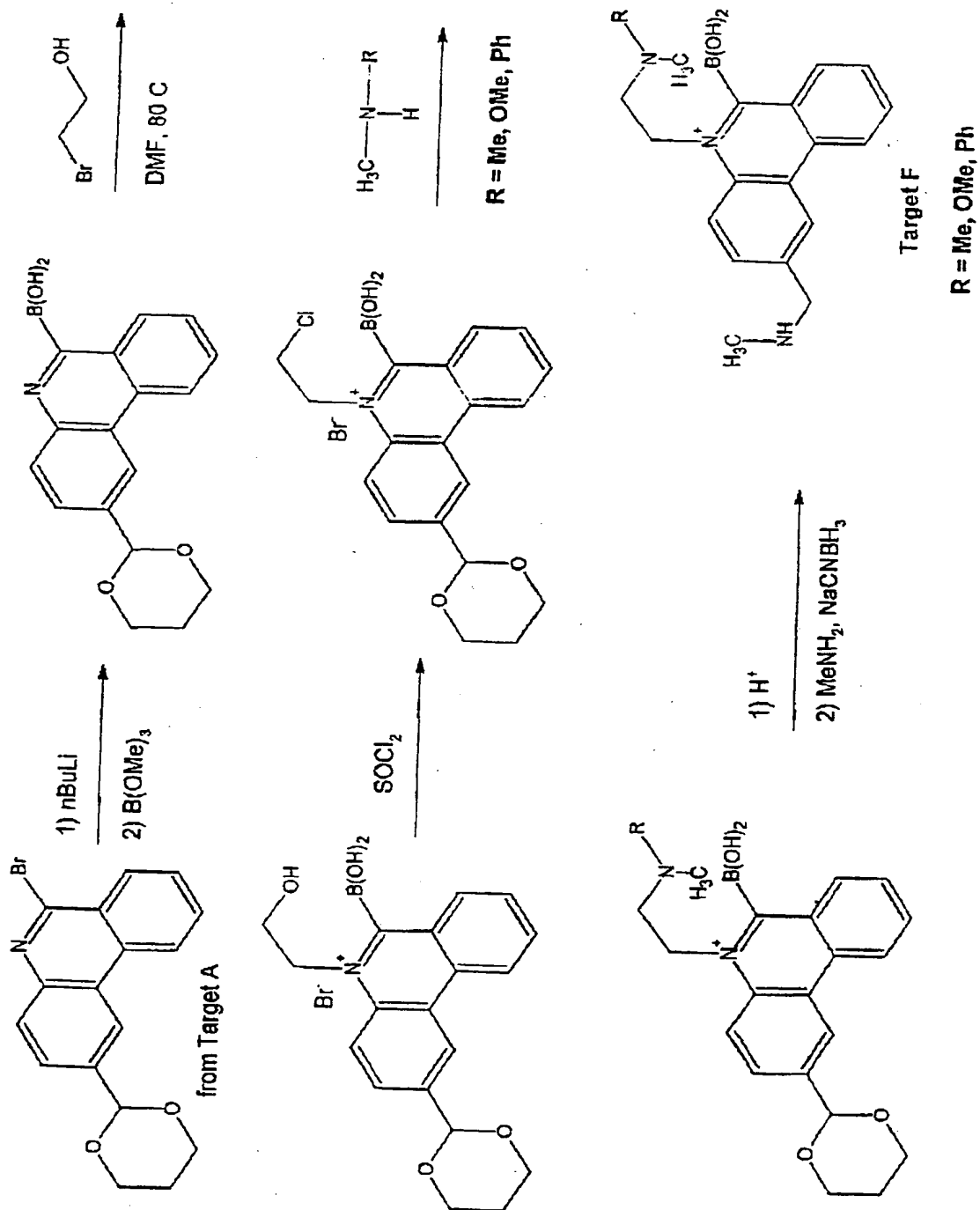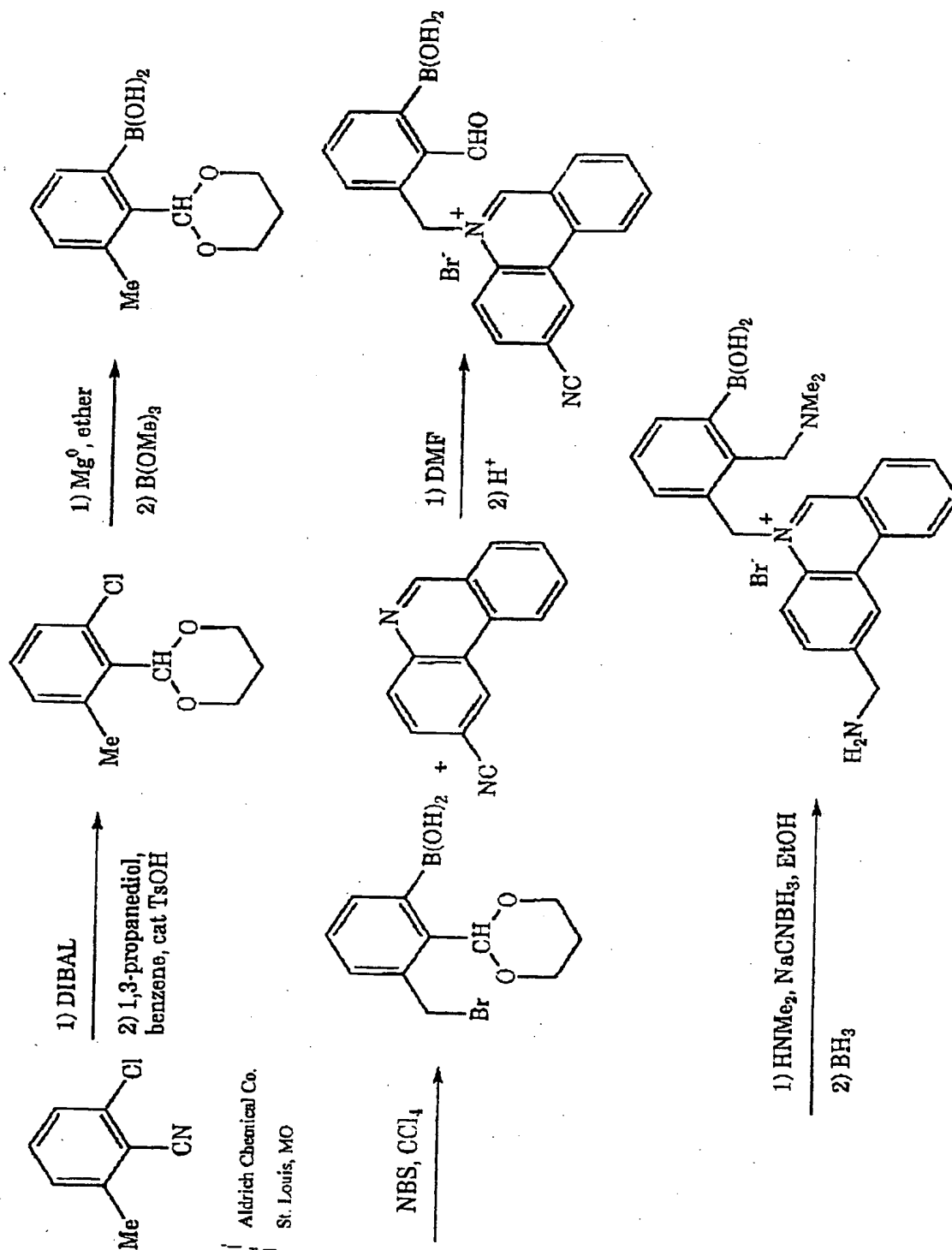
Synthetic Scheme for Approach 3

Synthetic Scheme for Approach 3, page 2

1) Br—CH$_2$—C(=O)—OtBu

2) TFA

H$_2$N-hydrogel, EDC, NHS
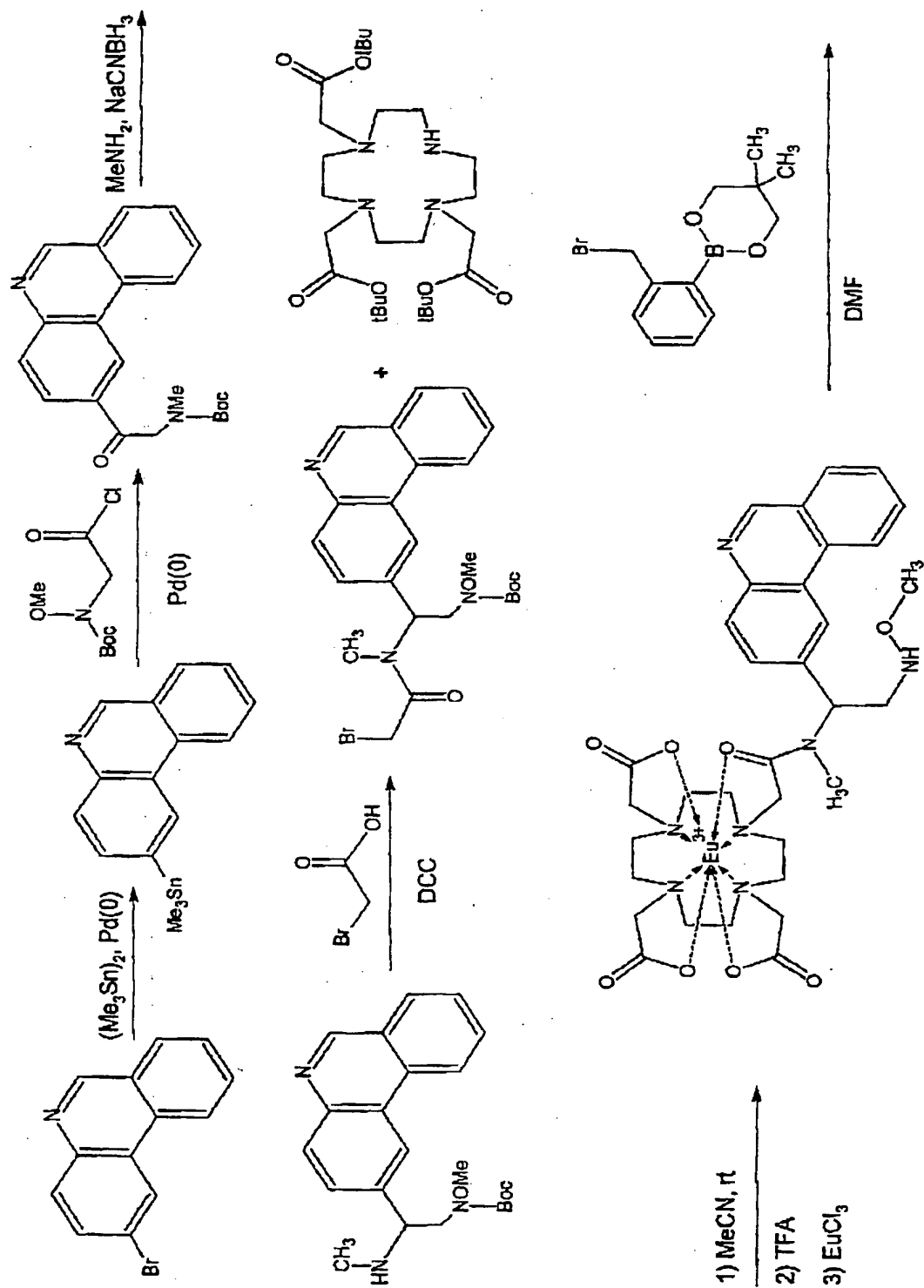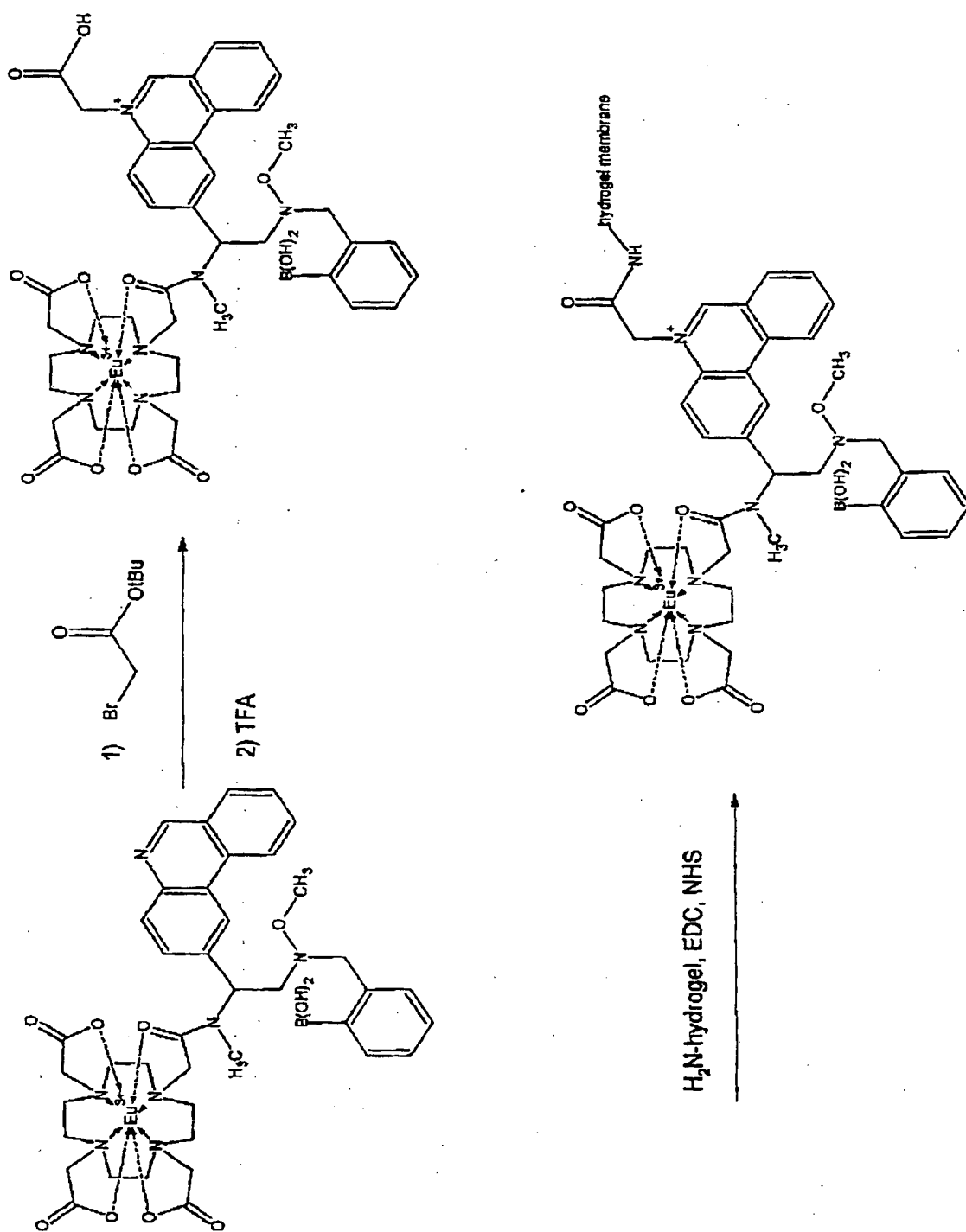
For additional details concerning aspects of the synthesis schemes depicted above, see the following publications, the contents of which are incorporated herein by reference:

5

1.   Walls, L. P., *JCS*, (1934), 104-109

2.   Reese, C. B., *JCS*, (1958), 895-901

3.   Muth, C. W. et.al., *J. Medicinal Chem*, (1973), Vol 16, No. 3, 1973

10   4.   Badger, G. M., et.al., *J.C.S.*, (1951), 3207-3211

5.   Ishiyama, T., et.al., *J. Org. Chem.* (1995), 60, 7508-7510

6.   Forrester, A. R., et.al., *J.C.S. Perkin I*, 612-615

7.   Petterson, R. C., et.al., *J. Org. Chem.*, (1974),

15   Vol. 39, No. 13, 1841-1845

8.   Nagarajan, K., et.al., *Indian Journal of Chemistry*, Vol. 11, Feb. 1974, 112-114

9.   Hollingsworth, B. L., et.al., *J. Chem. Soc.*, (1961), 3771-3773

20   10.   Finkelstein, J., et.al., *J. Amer. Chem. Soc.*, (1951), Vol 73, 302-304

11.   Parker, D., et.al., *J. Chem. Soc., Chem. Commun.*, (1997) 1777-78

12.   Stille, J. K., *Angew, Chem. Int. Ed. Engl.*, (1986),

25   Vol 25, 508-524

13.   Sherry, A. D., et.al., *Inorganica Chimica Acta*, (1987), Vol. 139, 137-139

14.   Bansal, N., et.al., *J. Magnetic resonance Imaging*, (1992) Vol. 2, 385-391

30   15.   Sherry, A. D., et.al., *J. Magnetic Resonance*, (1988), Vol 76, 528-533

The invention is illustrated by the following examples for further understanding thereof.

EXAMPLE I

Detection of Glucose and Fructose with
Eu(4-dibenzoylmethaneboronic acid)

5   25 µl of 6.5 mM Eu(4-dibenzoylmethaneboronic acid)$_4$
PyCl was added to 525 µl methanol and vortexed. From
stock solutions in methanol (4 µM, 400µM, 4mM and 40mM),
samples of glucose and fructose were separately prepared
at concentrations of 0.5 µM, 5 µM, 50 µM, 1 mM, 5 mM, 10

10   mM, and 20 mM for each of the two sugars. The results of
which are shown in Figure 9, the fluorescence emission
intensity of the Eu chelate complex at 613 nm was
monitored for each of the separate samples of glucose and
fructose after exciting the Eu chelate complex at the

15   required excitation wavelength of 365 nm.

EXAMPLE II

Synthesis of Europium Tetrakis –
5-naphthoyl-trifluoroacetoneboronic acid

20

I.   Preparation of 2,2-dimethylpropane-1,3-diyl-
      1-naphthylboronate (1)

The boronate group of the precursor is protected from

25   any potential adverse effects caused by subsequent
reaction conditions during synthesis of the europium
chelate complex according to the following procedure:

Naphthalene-1-boronic acid (15.2 grams, 0.0884 moles)

30   and 2,2-dimethyl-1,3-propanediol (10.0 grams, 0.0960
moles, 1.1 equivalents) were refluxed in toluene (200ml)
while removing water azeotropically using a Dean-Stark
trap for 28 hours. The toluene was then evaporated by
simple distillation, followed by aspirator pressure

35   distillation while heating for 2 hours until reaching a
temperature of about 80°C. The unreacted 2,2-dimethyl-

-65-

1,3-propanediol was then removed under vacuum (0.5mm)
while heating up to 60°C for 1 hour. A white solid
(20.94 grams, 99% purity) of 2,2-dimethylpropane-1,3-
diyl-1-naphthylboronate was obtained.

5

The product was verified by ¹HNMR (CDCl₃, 400MHz).

II. Friedel-Crafts Acetylation: Preparation of 2,2-
     dimethylpropane-1,3-diyl-5-acetyl-1-naphthylboronate
10   (2)

An acetyl group was introduced into the aromatic
structure of the precursor to form a diketone, according
to the following procedure:

15

2,2-dimethylpropane-1,3-diyl-1-naphthylboronate (1)
(21.0 grams, 0.0878 moles) was dissolved in 150 ml of dry
carbon disulfide while stirring in a 250 ml round bottom
flask in an ice water bath. Separate portions of
20   aluminum trichloride (28.7 grams, 0.215 moles) were added
over a period of two hours. The mixture was then stirred
and slowly warmed to room temperature over a period of
one hour. A sticky, dark, semisolid was seen deposited
inside the flask. The mixture was cooled again in an ice
25   water bath, whereafter a refluxing condenser was added to
the flask. Acetic anhydride (8.93 grams, 0.0875 moles)
was then added over a period of 2 hours. The mixture was
then warmed to 40°C to initiate the reaction. During
addition of the acetic anhydride, it was necessary to
30   agitate the reaction mixture (by hand swirling)
occasionally, as to control any exothermic reaction which
might occur during the reaction.

After allowing the reaction mixture to sit at room
35   temperature for 2 hours, the mixture was then heated

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slowly to 50°C over a period of 1 hour and maintained at that temperature for three hours. A dark solid was observed to form in the reaction mixture.

5   800 ml of ice water, 15 ml of concentrated hydrogen chloride and 250 ml of methylene chloride were then used together to decompose and extract the reaction mixture into two clear layers. The bottom organic layer was then collected, dried over sodium sulfate, and evaporated at
10   reduced pressure up to 80°C for three hours to yield 19.39 g of dimethylpropane-1,3-diyl-5-acetyl-1-naphthylboronate (2) in a semisolid form, having a yield of 78%.

15   III. Claisen Condensation: Preparation of 5-naphthoyl-
             trifluoroacetoneboronic acid (3)

      A β-diketone ligand was formed, as follows:

20   A mixture of sodium hydride was reacted with 2 ml of methanol (0.313 gram, 0.01302 moles) in 10 ml of dry ether. The resulting solution was dried under reduced pressure up to 100°C for two hours to yield sodium methoxide in a solid form. The sodium methoxide was then
25   treated with 45 ml of dry ether and cooled in an ice water bath. Ethyl trifluoroacetate (1.763 grams, 0.0124 moles) was then added, followed 10 minutes later by adding a solution of dimethylpropane-1,3-diyl-5-acetyl-1-naphthylboronate (2) (3.50 grams, 0.0124 moles) in 20 ml
30   of dry ether, which solution was added dropwise over a period of another 10 minutes. The mixture was then stirred for 30 minutes at room temperature and heated to reflux for 70 hours. While stirring the mixture in an ice water bath, 25 ml of water and 8 ml of 10% hydrogen

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chloride were added to acidify the water layer to a pH of
1. Two clear layers thereafter appeared in the flask.

5   The top etheral layer was then collected, dried over
sodium sulfate, and evaporated under reduced pressure up
to 60°C for 1 hour to yield the β-diketone in a dark
liquid form (4.36 grams). The resultant β-diketone was
purified by preparative silica gel TLC by eluting with
methylene chloride. The third band (Rf = 0.70-0.85) was
10   recovered from the TLC plate as a 25% yield of 5-
naphthoyl-trifluoroacetoneboronic acid (3) (1.20 grams).

The product was verified by proton NMR (400 MHZ)
spectra having a pattern characteristic of the enol form
15   of the β-diketone with peaks at sigma 6.69 (singlet) and
at sigma 15.28 (broad singlet).

IV.   Chelation/Complexation: Formation of the europium-
(beta-diketoneboronic acid) tetrakis complex (4)
20

The lanthanide chelate complex for use as a
fluorescent indicator molecule was then produced as
follows:

25   A solution of europium trichloride hexahydrate (0.7
mg, 0.0019 mmoles) in 0.5 ml absolute ethanol was added
to a solution of 5-naphthoyl-trifluoroacetoneboronic acid
(3) (2.2 mg, .0058 mmole) and piperidine (130 mg) in 0.5
ml of absolute ethanol. This mixture was heated slowly
30   to 70°C over a period of 2 hours and maintained at that
temperature for an additional 3 hours to form the
europium tetrakis complex (4).

The resultant solution exhibited a characteristic
35   orange-red emission upon irradiation with a hand-held,

long wave UV source. Additionally, a fluorescence
spectra, as measured with a Shimadzu fluorometer, showed
a peak excitation wavelength of 340 nanometers and an
emission pattern characteristic of a europium chelate
5   complex of 613 nanometers.

The invention has been described in connection with
certain preferred embodiments. Those skilled in the art
will recognize that modifications and improvements may be
10   made without departing from the spirit and scope of the
invention.

What is claimed is:

    1.   An indicator molecule for detecting the presence or concentration of an analyte, comprising a fluorescent lanthanide metal chelate complex having the formula:

$$M(--Ch(-R_x))_y$$

wherein:

M is a lanthanide metal ion; Ch is a chelator comprising a ligand; R is an analyte-specific recognition element and X represents the number of recognition elements R bound to each chelator; X=0 to 4, and Y=1 to 4; and the number of recognition elements R may be the same or different, provided that for one or more chelators, X>0; and wherein the presence or concentration of the analyte is detected by measuring any change in fluorescence emitted by the lanthanide metal chelate complex upon binding of the analyte to one or more chelators of the complex through the recognition element.

    2.   The indicator molecule of claim 1, wherein M of the lanthanide metal chelate complex is an europium ion or a terbium ion.

    3.   The indicator molecule of claim 2, wherein the ligand of the one or more chelators is an organic ligand comprising any one or more of a β-diketone or a nitrogen analog thereof, a dihydroxy, a carboxyl coordinating heterocycle, an enol, a macrobicyclic cryptand, a polyamino-polycarboxylic acid, a phenylphosphonic acid, an alkane or alkene group containing 1 to 10 carbon atoms, aromatic moieties, carbocyclic moieties and heterocyclic moieties.

    4.   The indicator molecule of claim 3, wherein the ligand of the one or more chelators comprises a moiety

selected from the group consisting of a β-diketone and a cyclen.

5.   The indicator molecule of claim 4, wherein the recognition element R of the lanthanide metal chelate complex comprises a boronate, arsenite or germanate group and the analyte is glucose.

6.   The indicator molecule of claim 1, wherein the one or more chelators further comprises a substituent for attaching the chelate complex to a solid support.
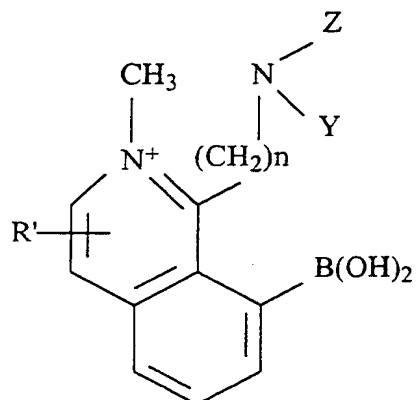
7.   The indicator molecule of claim 6, wherein the substituent for attaching the chelate complex to the solid support is a carboxyl, $-NH_2$ or $-OH$ group.

8.   The indicator molecule of claim 1, wherein the recognition element is selected from the group consisting of:

-71-
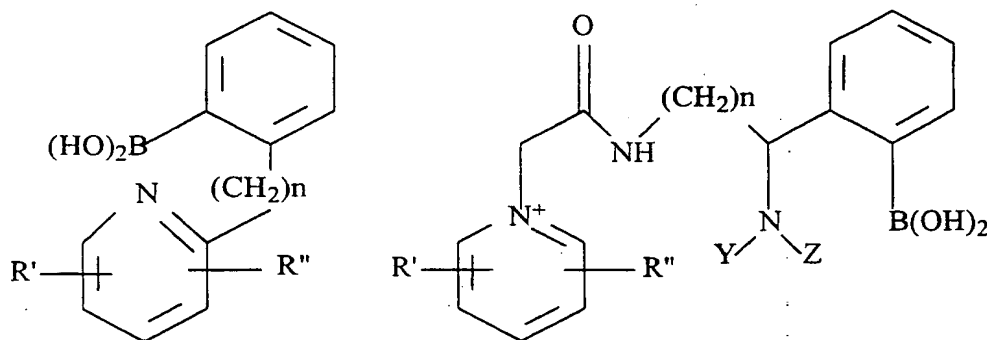
wherein n is 0, 1 or 2 in the right structure and 0 or 1 in the left structure;

5

wherein n is 1;

10
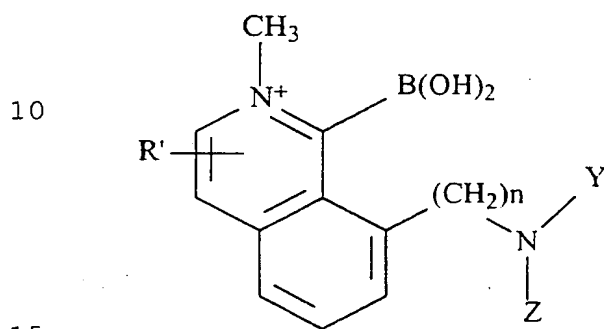
15

20

wherein n is 0 or 1;
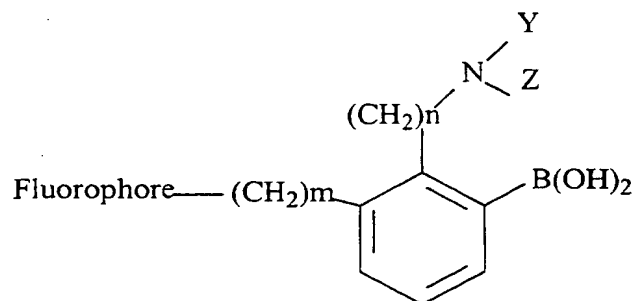
5

10

wherein n is 2;

15

wherein m is 0-5 and n is 1 or 2; and
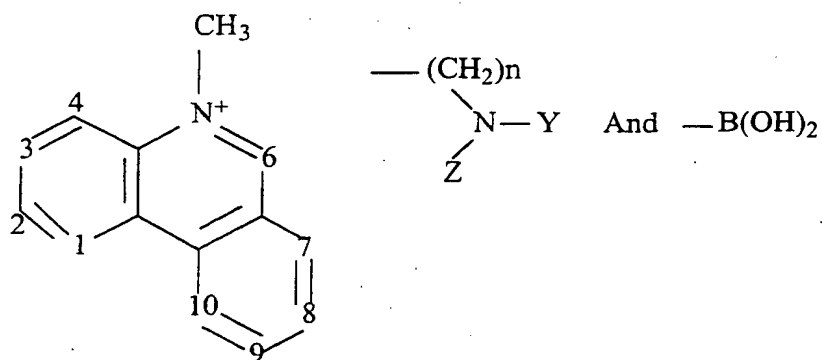
-73-

wherein n is 0 or 1, and the boronic acid and amine
substituents are located as a pair on positions 1 and 10,
3 and 4, 6 and 7, 7 and 8 or 9 and 10;
and all structures, where applicable, R' and R'' are each
independently fused aryl; aliphatic; primary, secondary
or tertiary amine; amide; carboxyl; ketone; ester;
alcohol; or aldehyde; and Y and Z are each independently
aliphatic, alkoxy or aryl); and derivatives thereof.

9. A fluorescent lanthanide metal chelate complex
for detecting the presence or concentration of an
analyte, having the formula:
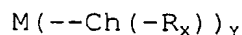
$$M(--Ch(-R_x))_Y$$

wherein:
M is a lanthanide metal ion; Ch is a chelator comprising
a ligand; R is an analyte-specific recognition element
and X represents the number of recognition elements R
bound to each chelator; X=0 to 4, and Y=1 to 4; and the
number of recognition elements R may be the same or
different, provided that for one or more chelators, X>0;

and wherein the presence or concentration of the analyte
is detected by measuring any change in fluorescence
emitted by the lanthanide metal chelate complex upon
binding of the analyte to one or more chelators of the
5   complex through the analyte-specific recognition element.

      10.   The fluorescent lanthanide metal chelate complex
of claim 9, wherein M is an europium ion or a terbium
ion.

10

      11.   The fluorescent lanthanide metal chelate complex
of claim 10, wherein the ligand of the one or more
chelators is an organic ligand comprising any one or more
of a β-diketone or a nitrogen analog thereof, a
15   dihydroxy, a carboxyl coordinating heterocycle, an enol,
a macrobicyclic cryptand, a polyamino-polycarboxylic
acid, a phenylphosphonic acid, an alkane or alkene group
containing 1 to 10 carbon atoms, aromatic moieties,
carbocyclic moieties and heterocyclic moieties.

20

      12.   The fluorescent lanthanide metal chelate complex
of claim 11, wherein the ligand of the one or more
chelators comprises a β-diketone or a cyclen.

25   13.   The fluorescent lanthanide metal chelate complex
of claim 12, wherein the recognition element of the
lanthanide metal chelate complex comprises a boronate,
arsenite or germanate group and the analyte is glucose.

30   14.   The fluorescent lanthanide metal chelate complex
of claim 9, wherein the recognition element is selected
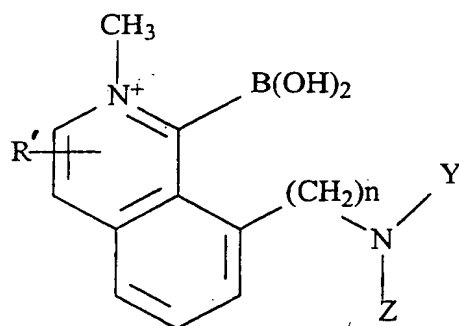from the group consisting of:
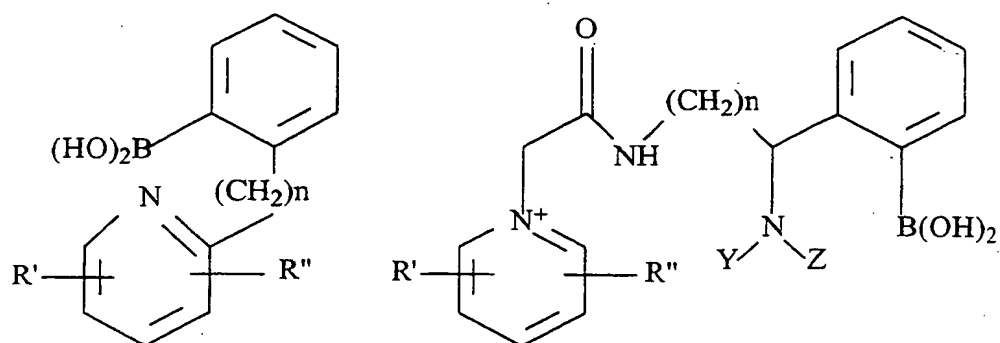
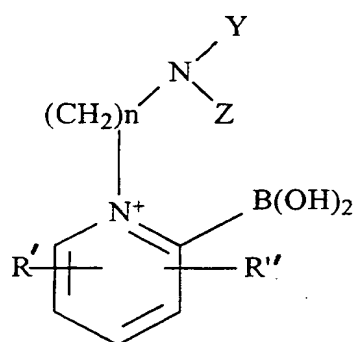wherein n is 0, 1 or 2 in the right structure and 0 or 1 in the left structure;
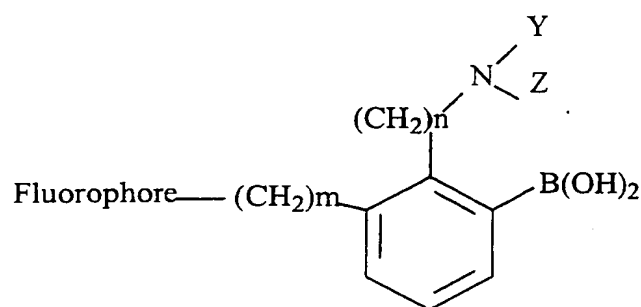
5
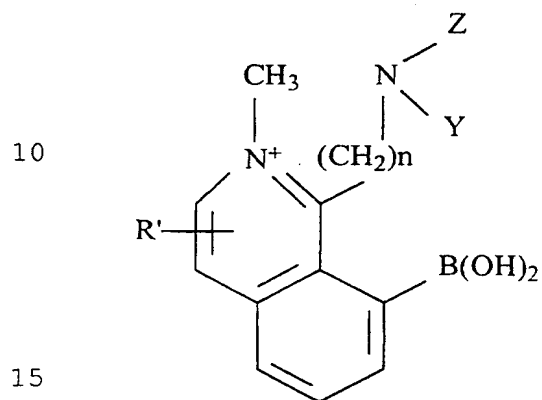
10

15

wherein n is 1;

wherein n is 0 or 1;

5

wherein n is 2;
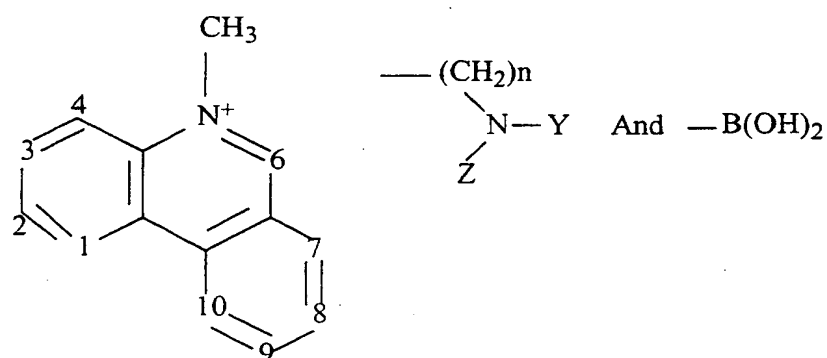
-77-

$$\text{Fluorophore} — (CH_2)m \overset{\overset{\displaystyle (CH_2)n}{|}}{\bigcirc} B(OH)_2$$

with

$$\overset{Y}{\underset{Z}{N}}$$

on the (CH_2)n chain.

wherein m is 0-5 and n is 1 or 2; and

5

$$\text{—}(CH_2)n \overset{Y}{\underset{\underset{Z}{|}}{N}} \quad \text{And} \quad \text{—}B(OH)_2$$

wherein n is 0 or 1, and the boronic acid and amine
substituents are located as a pair on positions 1 and 10,
3 and 4, 6 and 7, 7 and 8 or 9 and 10;
and all structures, where applicable, R' and R'' are each
10   independently fused aryl; aliphatic; primary, secondary
or tertiary amine; amide; carboxyl; ketone; ester;
alcohol; or aldehyde; and Y and Z are each independently
aliphatic, alkoxy or aryl);
and derivatives thereof.

-78-

15.   A method for detecting the presence or
concentration of an analyte in a sample, comprising the
steps of:

a)   exposing the sample to an indicator molecule

5   comprising a fluorescent lanthanide metal chelate complex
having the formula:

$$M(--Ch(-R_x))_Y$$
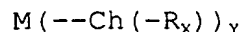
wherein:

10   M is a lanthanide metal ion; Ch is a chelator comprising
a ligand; R is an analyte-specific recognition element
and X represents the number of recognition elements R
bound to each chelator; X=0 to 4, and Y=1 to 4; and the
number of recognition elements R may be the same or

15   different, provided that for one or more chelators, X>0;
and

b)   measuring any change in fluorescence emitted by
the lanthanide metal chelate complex upon binding of the
analyte to one or more chelators of the complex through

20   the analyte-specific recognition element, thereby
detecting the presence or concentration of the analyte.

16.   The method of claim 15, wherein M of the
lanthanide metal chelate complex is an europium ion or a

25   terbium ion.

17.   The method of claim 16, wherein the ligand of
the one or more chelators of the lanthanide metal chelate
complex is an organic ligand comprising any one or more

30   of a β-diketone or a nitrogen analog thereof, a
dihydroxy, a carboxyl coordinating heterocycle, a
macrobicyclic cryptand, a polyamino-polycarboxylic acid,
an enol, a phenylphosphonic acid, an alkane or alkene
group containing 1 to 10 carbon atoms, aromatic moieties,

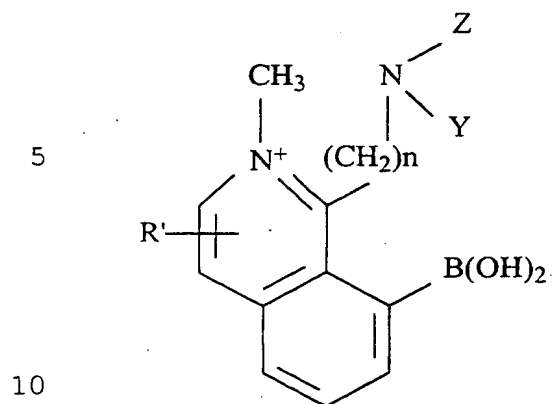35   carbocyclic moieties and heterocyclic moieties.

-79-

18.   The method of claim 17, wherein the ligand of
the one or more chelators comprises a β-diketone or a
cyclen.

5   19.   The method of claim 18, wherein the recognition
element R of the lanthanide metal chelate complex
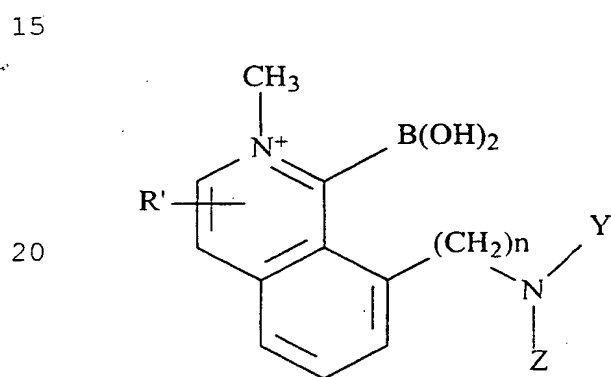comprises a boronate, arsenite or germanate group and the
analyte is glucose.

10   20.   The method of claim 15, wherein the recognition
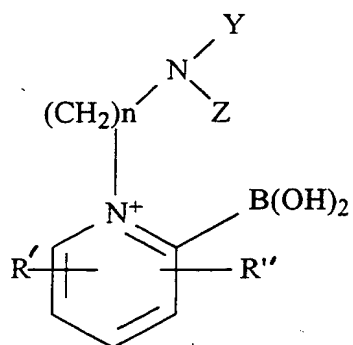element is selected from the group consisting of:
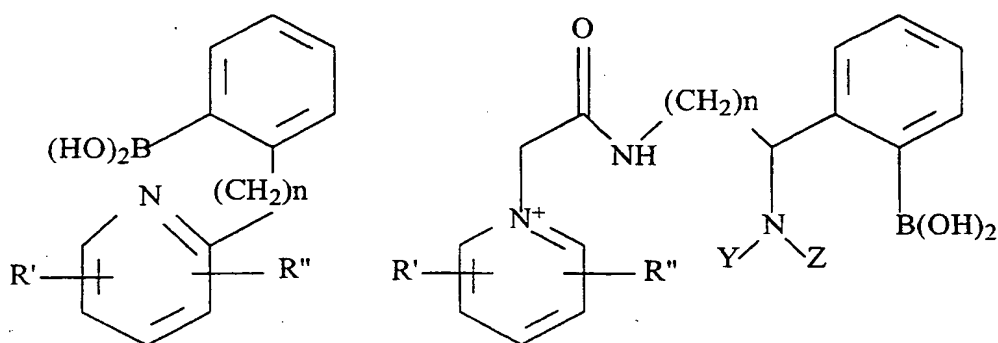
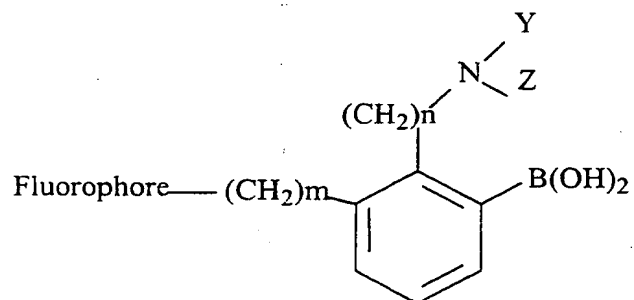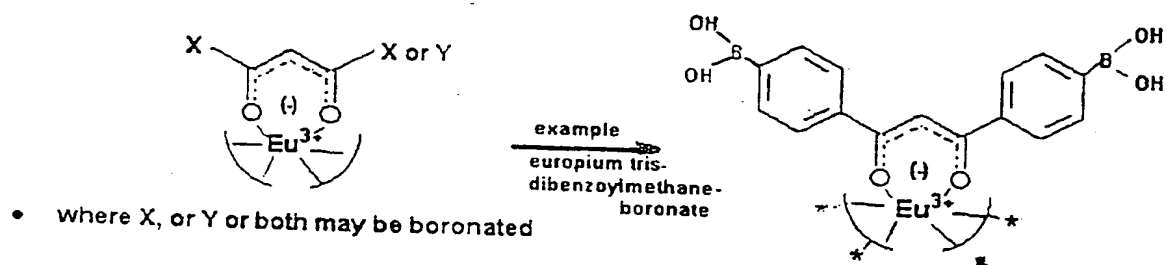15   wherein n is 0, 1 or 2 in the right structure and 0 or 1
in the left structure;

5

10

wherein n is 1;

15

20

25

wherein n is 0 or 1;

$$
\begin{array}{c}
Y \\
\mid \\
N \\
\diagup \quad \diagdown \\
Z
\end{array}
$$

(CH$_2$)n

R' — [pyridinium ring, N$^+$] — B(OH)$_2$

R''

wherein n is 2;

$$
\begin{array}{c}
Y \\
\mid \\
N \\
\diagup \quad \diagdown \\
Z
\end{array}
$$

(CH$_2$)n

Fluorophore —— (CH$_2$)m — [benzene ring] — B(OH)$_2$

wherein m is 0-5 and n is 1 or 2; and

5

CH$_3$

[fused bicyclic ring system with N$^+$, positions labeled 1, 2, 3, 4, 6, 7, 8, 9, 10]

—(CH$_2$)n

N — Y   And   —B(OH)$_2$

Z

-82-

wherein n is 0 or 1, and the boronic acid and amine
substituents are located as a pair on positions 1 and 10,
3 and 4, 6 and 7, 7 and 8 or 9 and 10;
and all structures, where applicable, R' and R'' are each
5   independently fused aryl; aliphatic; primary, secondary
or tertiary amine; amide; carboxyl; ketone; ester;
alcohol; or aldehyde; and Y and Z are each independently
aliphatic, alkoxy or aryl);
and derivatives thereof.

10

Figure 1

Boronic Acid-Containing Europium Chelate for
Glucose Detection

- where X, or Y or both may be boronated

Figure 2

Figure 3

Figure-4

Ex=358nm
Em=613.5nm

no catechol

catechol added

Time (microseconds)

Intensity

x10³

Figure 5

Fig. 6

Fig. 7

Fig. 8

Eu(DBM) vs. Eu(Boronated DBM) with glucose and fructose titration
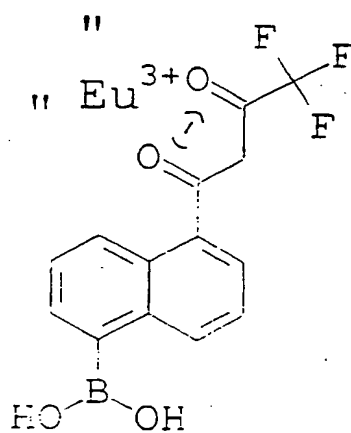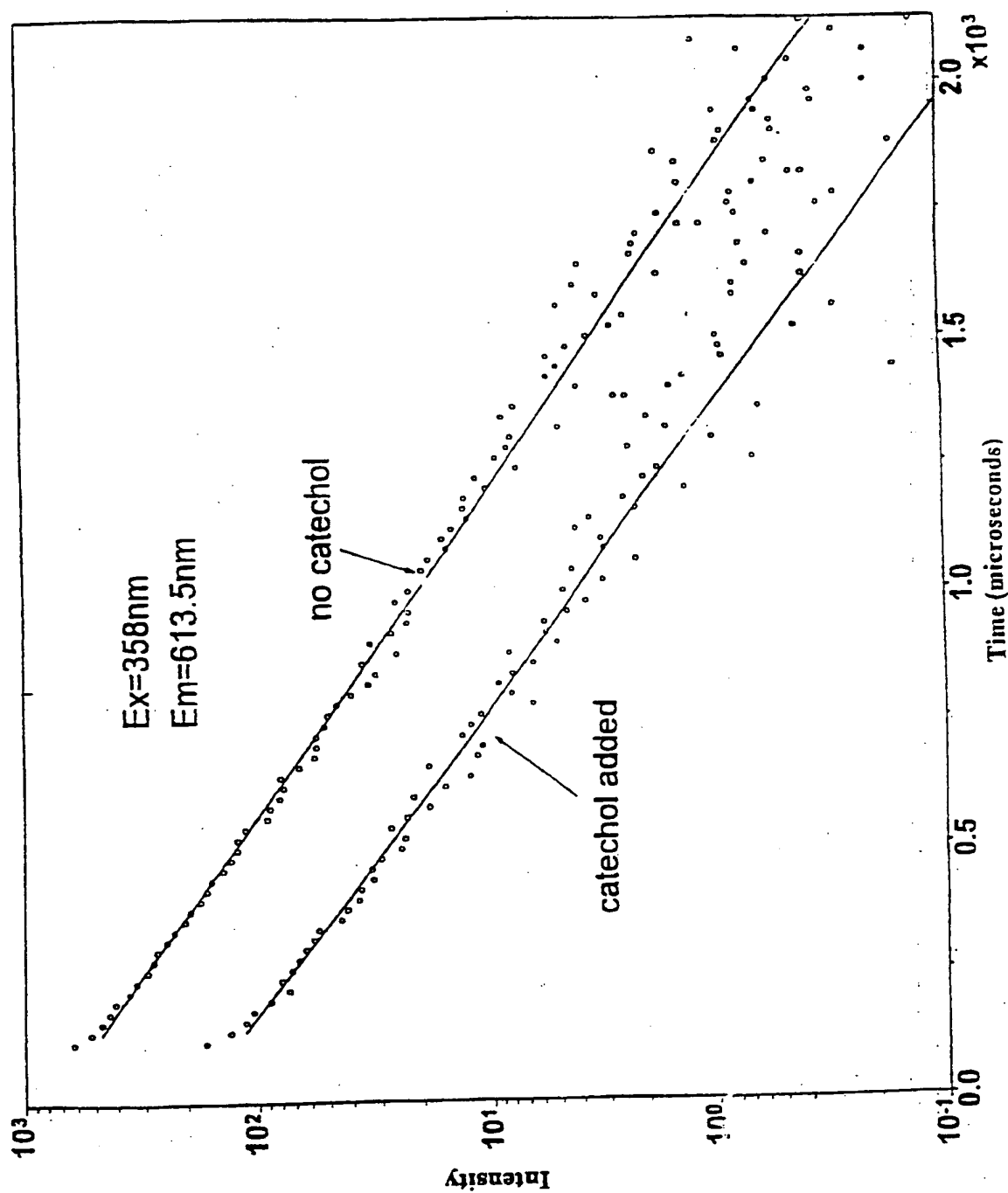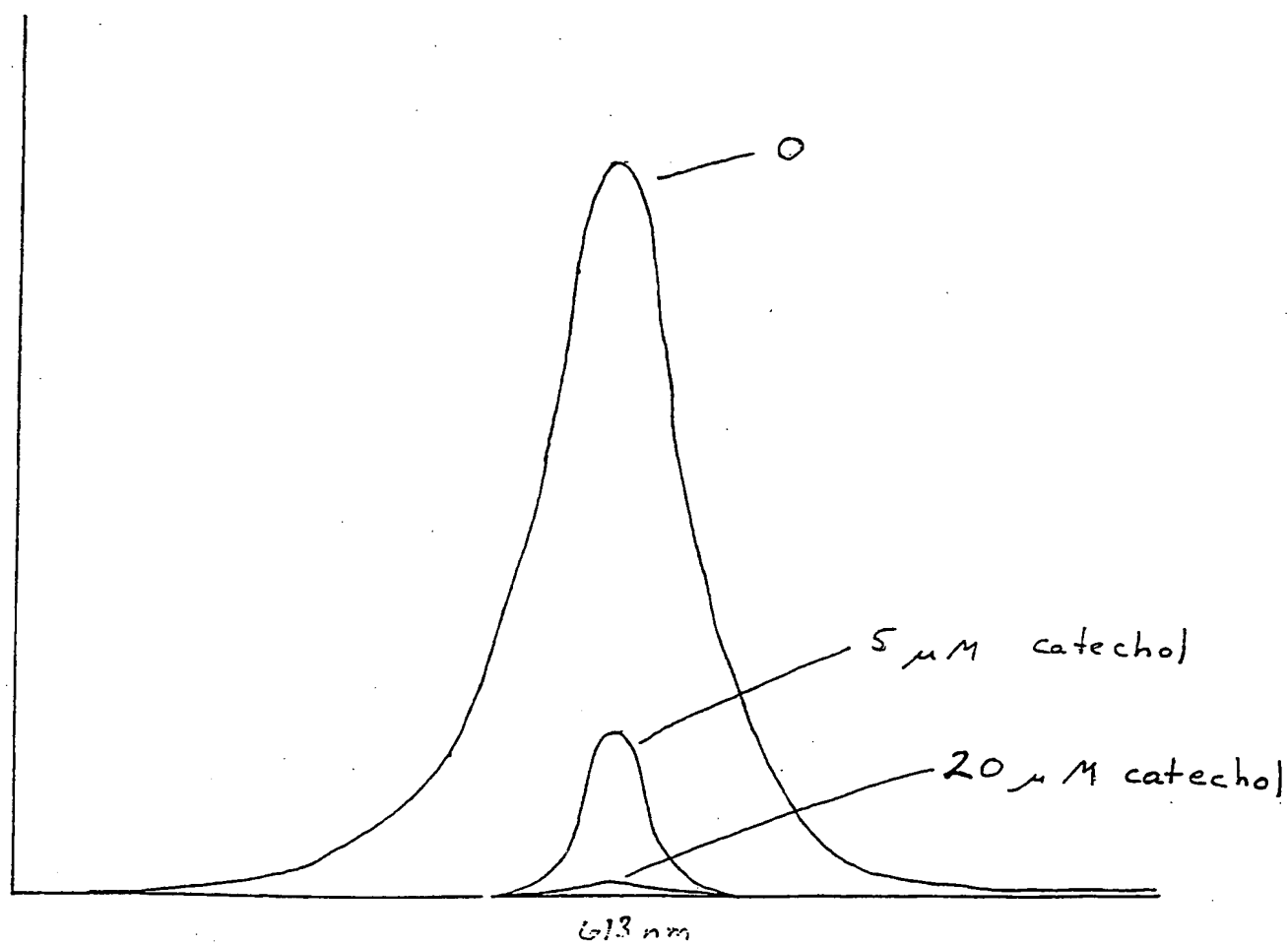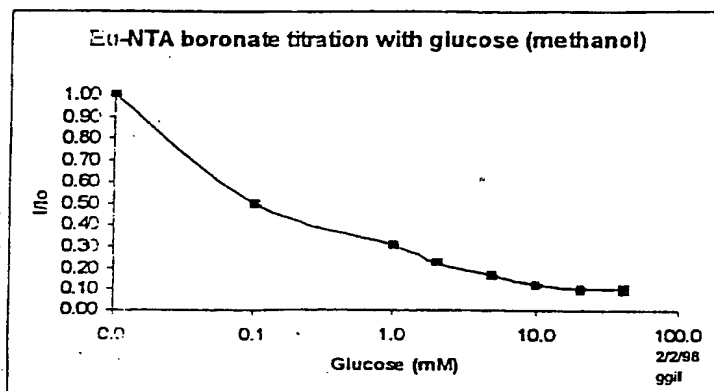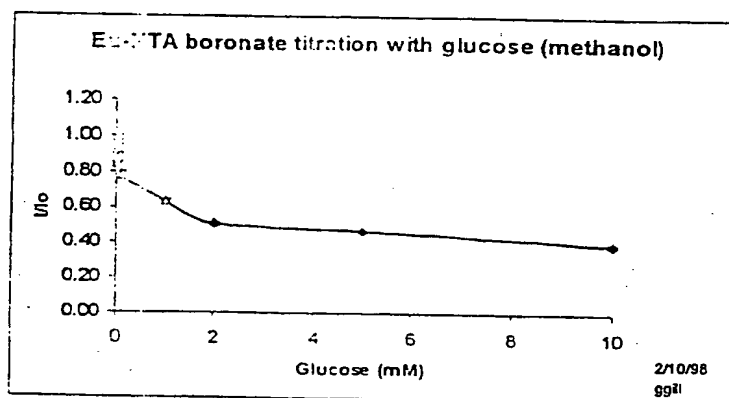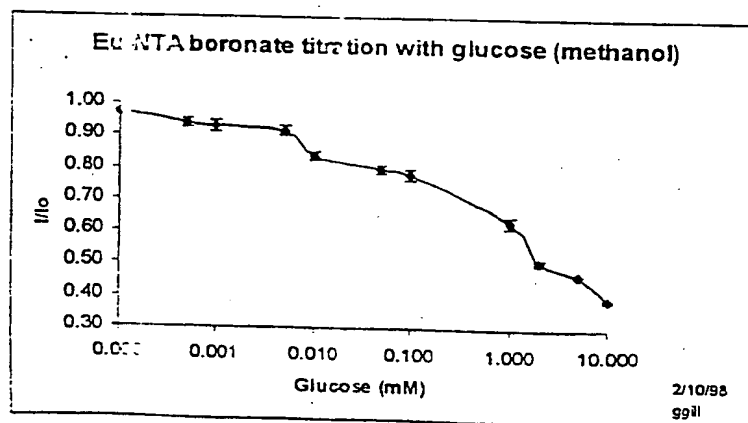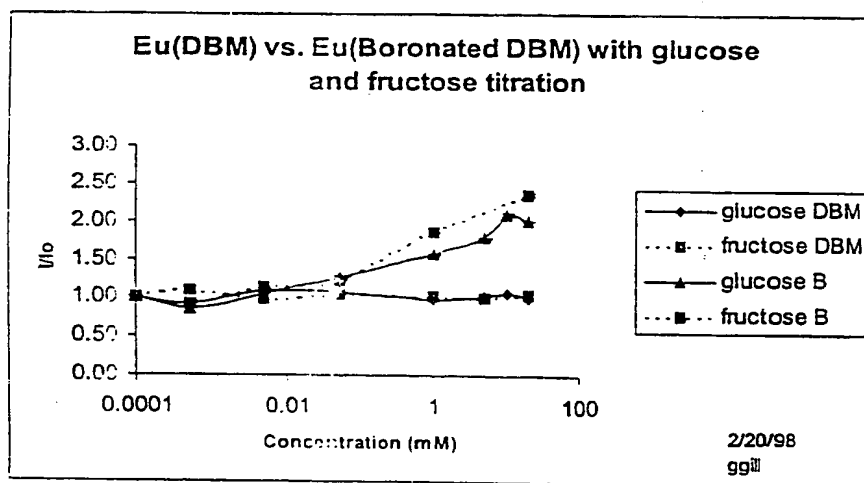
Figure 9

Figure 10

Europium Beta-Napthoyltrifluoroacetone-Boronate (Eu-BNTAB)

Eu-NTA boronate titration with glucose (methanol)

2/2/98
ggill
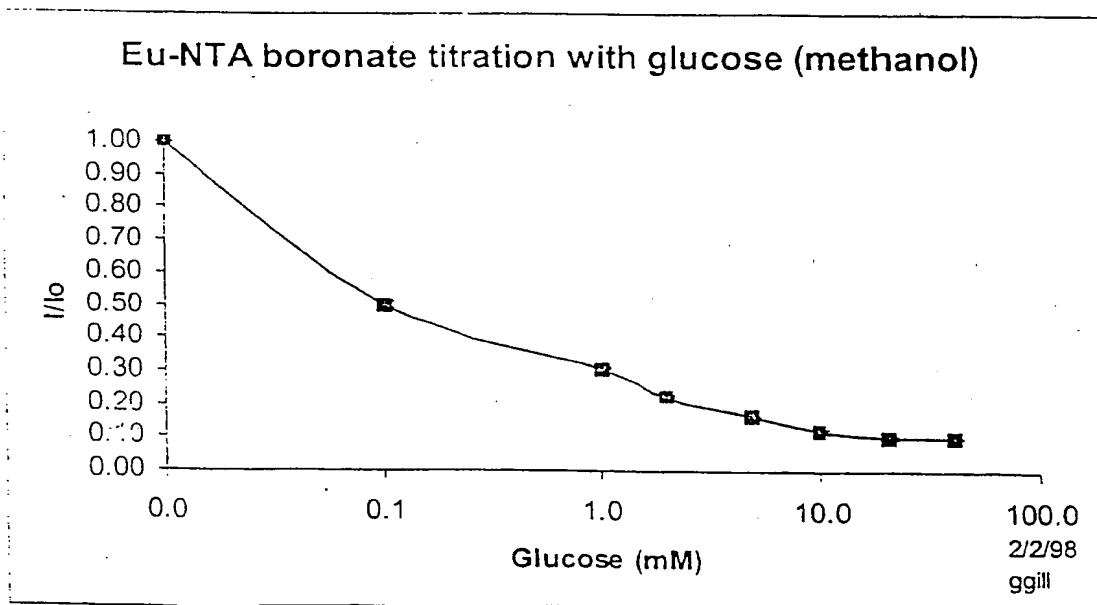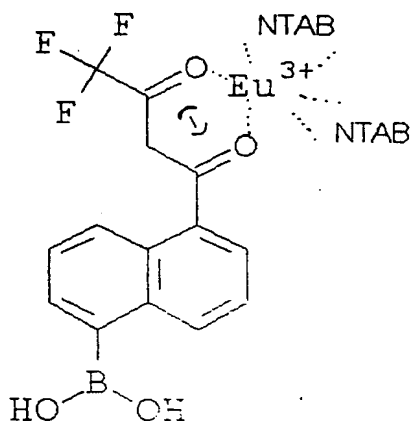
Figure 11

Europium Dibenzoylmethane Boronate (Eu-DBMB)
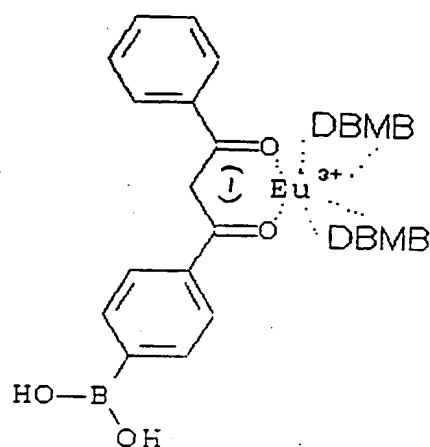
Eu(dibenzoylmethaneboronic acid) with glucose
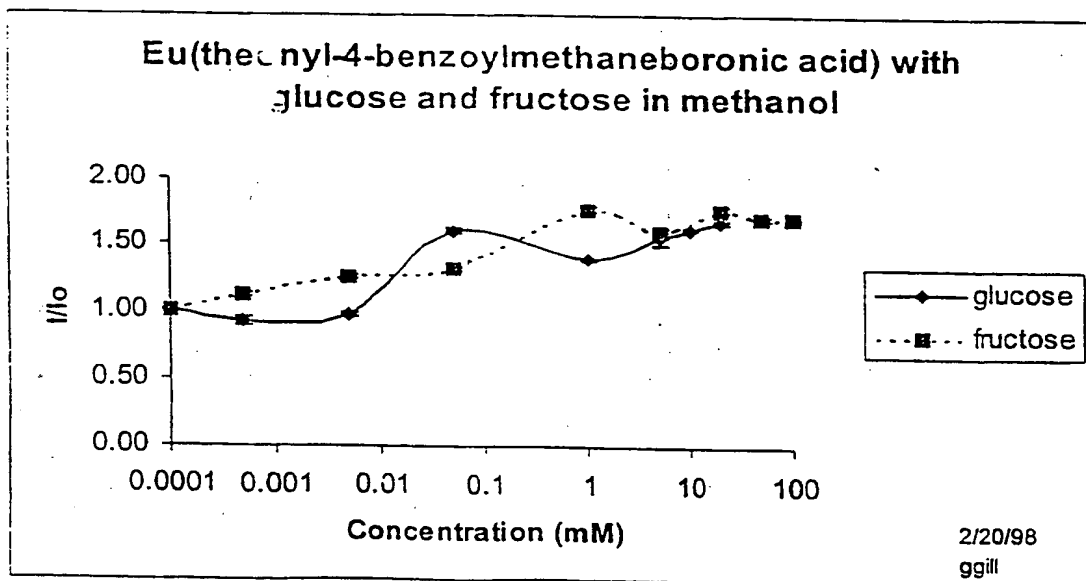and fructose in methanol
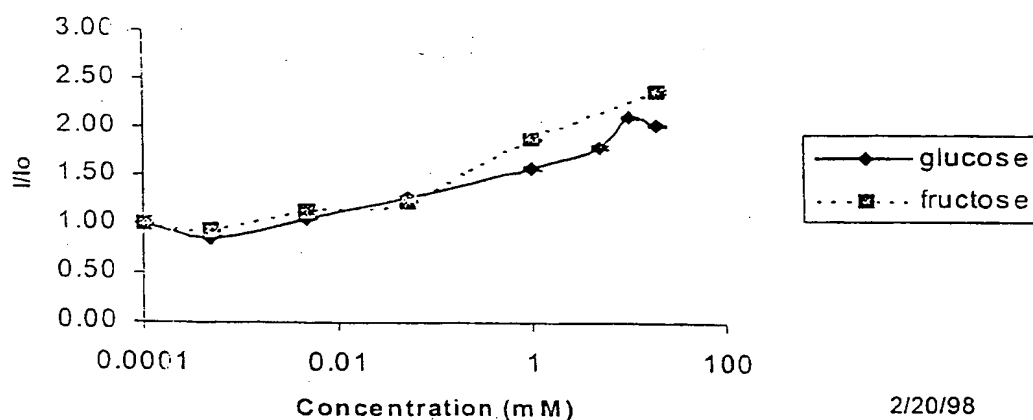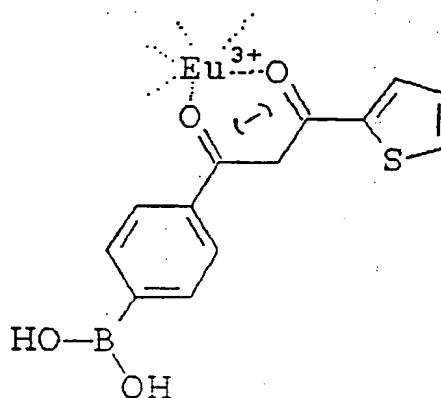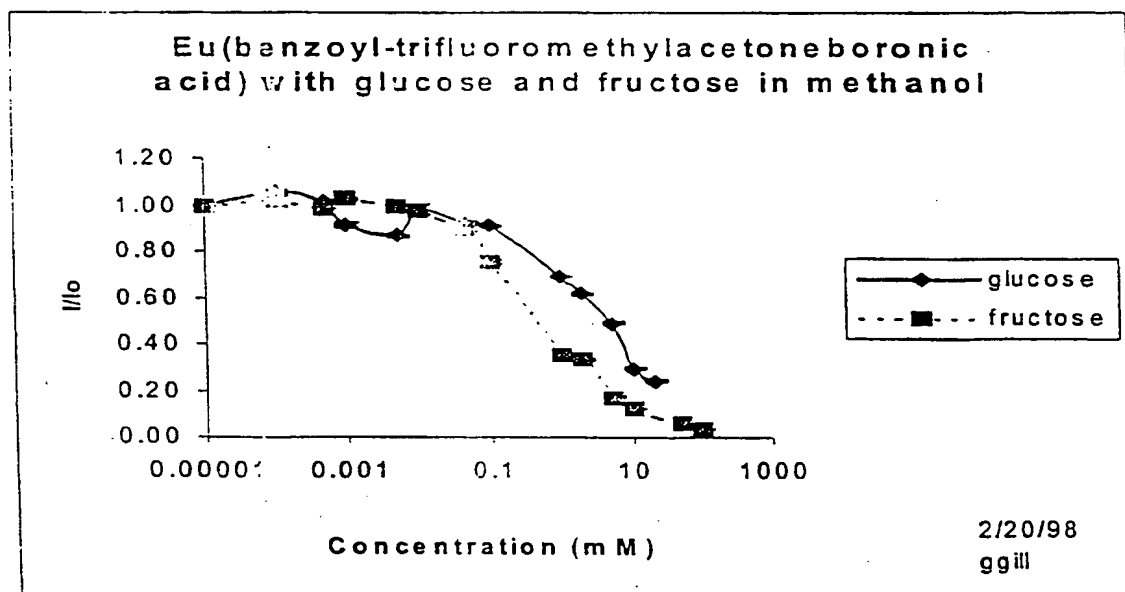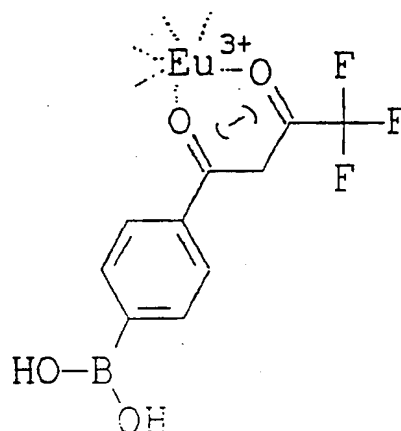

2/20/98
ggill

Figure 12

Europium Tetrakis Theonyl -4- Benzoyl Methane Boronate

Eu(theonyl-4-benzoylmethaneboronic acid) with glucose and fructose in methanol

2/20/98
ggill

Figure 13

Europium Tris-Benzoyl Trifluoro Acetone Boronate (Eu-BTAB)

Eu(4-benzoyl-trifluoromethylacetoneboronic acid), PyCl – glucose concentration varies from 0, .0001, .0005, .001, .005, .01, .05, .1, 1, 2, 5, 10, and 20 mM. Fructose concentration varies from 0, .0001, .0005, .001, .005, .01, .05, .1, 1, 2, 5, 10, 50 , and 100 mM.